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## Contents

Studies of Certain Phases of the Interrelationship between Soil and Plant: I. Availability of Mineral Plant Nutrients in Relation to the Degree of Dispersion. WALTER THOMAS.....	249
Contribution to the Chemical Composition of Peat: III. Chemical Studies of Two Florida Peat Profiles. SELMAN A. WAKSMAN AND KENNETH R. STEVENS.....	271
Microbiological Activities in the Soil of an Upland Bog in Eastern North Carolina. IVAN V. SHUNK.....	283
The Effect of Moisture Content and Cropping on Exchangeable Calcium and Magnesium, with Particular Reference to Rice Soil. W. H. METZGER.	305
Some Influences of the Development of Higher Plants upon the Micro- organisms in the Soil: I. Historical and Introductory. ROBERT L. STARKEY.....	319

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## SOIL SCIENCE



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# STUDIES OF CERTAIN PHASES OF THE INTERRELATIONSHIP BETWEEN SOIL AND PLANT: I. AVAILABILITY OF MINERAL PLANT NUTRIENTS IN RELATION TO THE DEGREE OF DISPERSION<sup>1</sup>

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## A. INTRODUCTORY

The subject matter of this paper deals with a field of research that, although of the greatest importance, is still in a state of flux. Notwithstanding the existence of a voluminous literature on the permeability to and absorption of substances by living animal and plant cells, there is little exact knowledge. And to the complex of factors now to be considered that of colloidal behavior must be added.

Colloidal solutions have been *arbitrarily* defined on the basis of the size of the dispersed particles ( $1\mu$  to  $100\text{--}200\mu$ ). There is, however, no infallible test for colloidality. Even the ultra microscope and cataphoresis tests may fail under some conditions and with some colloids (82). Dialysis experiments, moreover, may not always be specific, for artificial semi-permeable (collodion) membranes have been prepared that are permeable to one crystalloid (e.g., NaCl), but not to another (e.g.,  $\text{NaNO}_3$ ) (7, 8); to starch and dextrin, but not to night blue and other colloidal dyes; to colloids but not to crystalloids (45, 46).

### 1. Theories of permeability

It is not definitely known what makes a membrane semi-permeable. According to Kahlenberg (45) an absolutely semi-permeable membrane exists only in theory. From their comprehensive studies on the conditions governing the permeability of certain physiological membranes for various *electrolytes*, Michaelis and Weech (59) conclude that it is fallacious to speak of a *definite permeability* of any type of membrane for electrolytes, since in their experiments the behavior towards the passage of electrolytes was found to depend on a variety of conditions. Thus, these investigators showed that by changing the character and magnitude of the force driving the ions across the membranes

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and, according to the history of previous treatment of the membrane, the whole character of the so-called specific permeability for ions of the membrane could be varied *without any substantial change in the structure, chemical composition or pore size of the membrane*. And Höber (39) is of the opinion that membranes possess both a "physiological" as well as a "physical" permeability, because the mechanism of penetration into membranes by the different classes of substances, such as organic substances, salts, acids, bases and water, appear to be so distinctly different with each group. The older definitions, therefore, of semi-permeable membranes break down entirely.

All theories of the permeability of *living* cells assume the existence of some sort of a "membrane," although differences of opinion exist as to its nature. The more prominent of the older conceptions of the factors determining the penetration of substances into living cells are Overton's "lipoid" theory (68) and Clowes' conception of the cell membrane as an "emulsion" (13); but it has been urged (41) that no one theory is capable of explaining all the peculiarities of cell permeability such as, for example, the penetration of water-soluble substances like sugar and which are not lipoid-soluble. Moreover, most of the theories break down in accounting for functional changes in permeability, that is, the permeability of the plant cell is not constant. Nevertheless, most authorities agree that there can be little doubt that "lipoid" solubility is a property favoring ready entrance into living cells, the sole exception to the rule known at present being the cases presented by a *colloidal dye* in the presence of plant cells (39).

## 2. What constitutes true colloidal behavior?

One of the main difficulties to be faced in an evaluation of the factors determining whether or not colloids can be absorbed by plants is in characterizing what constitutes true colloidal behavior. Although colloids have been arbitrarily defined on the basis of the size of the dispersed particles, there is no agreement on what constitutes true colloidal behavior. Loeb (55) premises that ampholytes like the proteins show colloidal behavior only when the protein ions are prevented from diffusing through those membranes or gels that are readily permeable to the ions of ordinary salts and that the distinction between crystalloids and colloids, as Graham (29) conceived the difference, is not tenable. If all ampholytes behave like proteins (as doubtless they do), then, according to Loeb's view, they are true colloids only at the isoelectric point, i.e., when they exist as non-ionic substances. The ions of Loeb, then, that show true colloidal behavior are the same as the micellae of McBain that originate through the electrolytic dissociation of neutral colloidal systems.

If, as it would appear, colloidal behavior is a surface phenomenon, the clearest conception of the behavior of colloids is obtained, as Wilson suggests (96), by regarding the particles as having more of their valences directed from the molecule and, therefore, unsatisfied (i.e., residual valency). The consequence of the existence of such free valences will be to cause substances

approaching their surfaces to combine and give up their charge to the colloid. The colloid may also acquire its charge by ionizing itself (22).

As Oakley (65) well expresses it, the colloidal condition may well be conceived as a compromise due to the differences in the affinity of the un-ionized and ionized groups for water, the boundary of the ionized ions being conditioned by the balance existing between the osmotic and electrometric forces. The membrane defined by the limits of this boundary will be governed by the Gibbs-Donnan equilibrium, the non-diffusible ions being the anions or cations that are partners to the colloidal cations or anions, resulting in a potential difference (P.D.) between the surface layers and the bulk of solution. This P.D. is found by equating the electrical work done by moving one equivalent of ions across the membrane to the work done against osmotic pressure, i.e., to  $\frac{R \cdot T}{F} \log y/x$ , where  $y/x$  are the concentrations of the negative ions on the two sides of the membrane.

### 3. On dialysis experiments

But all of these, conceptions, of colloidal behavior fail to explain much that is pertinent to our problem. For example, does dialysis increase or decrease the stability of sols? Hardy (35) maintains that dialysis as a means of isolating unstable or insoluble hydrous colloidal matter is open to criticism on the grounds that it progressively removes adsorbed stabilizing (peptizing) ions, thus destroying equilibrium conditions. The experiments of Thomas and Frieden (83) furnish an excellent example of this. There is a wide gap in our knowledge as to the causes operative in producing stability of sols and, therefore, of their exact composition. Take ferric hydrosol as an example. Thomas and Frieden (83) claim that the ferric oxide sol is represented by the formula  $x\text{Fe}_2\text{O}_3$ ,  $y\text{FeCl}_3$ , and that it exists in solution as the complex ions  $x\text{Fe}_2\text{O}_3 \cdot \text{Fe}^+$  and  $\text{Cl}^-$ . But additional corroborative evidence is lacking to support this view. Indeed, Sorum's investigations (78) established beyond much doubt that stable hydrosols giving no test for  $\text{Cl}^-$  can exist. From Beans and Eastlack's work (3) it might be deduced that the ferric oxide sol in Sorum's experiments is stabilized by the small amount of Fe in solution (for the oxide is soluble to the extent of 1 p.p.m.), or, possibly, it might be stabilized by the hydrogen ion from the ionization of the water on its surface.

From the foregoing it will be realized how fragmentary is our knowledge of the facts of permeability and how impossible it is in the present state of our knowledge to formulate any immutable laws of membrane action. This condition, however, may not prevent us from critically examining in detail the available experimental evidence bearing on our problem, to weigh the facts and to interpret them according to the scientific method. This we shall proceed to do.

## B. EXPERIMENTS WITH ARTIFICIAL MEMBRANES, ISOLATED CELLS OR TISSUES AND ABNORMAL PLANTS

### 1. Artificial membranes

*a. Gurchot's theory of colloidal penetration.* The suggestive researches of Gurchot (31), indicating the mechanism of the reversible permeability of copper ferrocyanide membranes, have given additional information on the possible mechanism of penetrability. His copper ferrocyanide membranes give all evidences of behaving as a dynamic system. He concludes that the whole question of the action of his membranes involves adsorption, coagulation and peptization. That some form of adsorption, or, perhaps, solution of the septum takes place is now widely accepted. This is the basis of Overton's theory (67) and is very strikingly demonstrated in Kahlenberg's experiments (45, 46) with rubber damask and lanoline silk membranes and in Overton's own experiments (67) on animal and plant cells.

It is possible that a satisfactory working hypothesis of the behavior of *artificial* membranes may be worked out in the future on the basis of reduction or neutralization of the charge upon their colloidal surfaces. Penetration of the membrane by colloids would, if such a theory is substantiated, be due to membrane coagulation due to selective adsorption as a result of differences in potential set up at the interface between the solid and liquid phases in accordance with the Gibbs-Donnan law. It would serve to explain why substances pass through a membrane at certain times that do not do so normally. Gurchot's theory of membrane action does not imply that penetration is always accomplished by means of selective adsorption and coagulation. On the contrary, as he points out, under some conditions a membrane may act like a sieve for salts in dilute solution, because in this case they will, if the solutions are dilute enough, go through the membrane without coagulating it. This is entrance by osmosis. But when the concentration is raised the charge of the membrane will, if the cation is absorbed more than the anion, be neutralized and coagulation will, therefore, follow, in which case the salts or ions pass through by diffusion as through a dialyzing membrane. Indeed, Weech and Michaelis (92) are disposed to account for the difference in the diffusion rates of the non-electrolytes examined by them to the varying sizes of the pore channels of the membranes.

From Gurchot's results it can scarcely be questioned that artificial membranes, like the potassium ferrocyanide septums used in his experiments, may, under certain conditions, permit the diffusion of colloids; but the experimental demonstration and proof of colloid penetration in the case of normal living cells, as will be shown, is less convincing.

*b. Equilibrium of colloidal and molecularly dispersed phases.* There is, moreover, another possible mechanism that must not be overlooked. If, as it is reasonable to suppose from the hydration theory (96), that a fractional portion of all sols are in true molecular solution, penetration of colloids into

cells would take place, preferably by the entrance of that portion of the substance which is in true solution. The disturbance of the equilibrium produced by the diffusion of solute would enable more of the colloid to go into true solution. Evidence for this view is supported by the work of Foerdi and also of Fredenthal as recorded by Bechhold (4). From the observation that after ultrafiltering pure starch "solutions" a given fraction of the "solution" always passed through his membranes, the former concluded that for every concentration of the starch solution a balance existed between the larger particles and the molecularly dissolved starches. This view is upheld by Fredenthal who found that soluble starch produced a definite lowering of the freezing point (F.P.). We shall have occasion to discuss this conception later. But the experiments cited cannot be regarded as final, for marked differences existing in the membranes used by different investigators might vitiate comparisons unless the results are expressed in terms of "diffusion coefficients" (92). There is little doubt that differences in structure and composition of membranes, together with factors already stated (59) have contributed not a little to the difficulties presented in interpreting much of the experimental work on the ions present in the soil solution and which have been based upon dialysis experiments with collodion membranes. The confusing results on aluminum as a factor in soil acidity may be cited as an example (21, 43, 57).

## 2. Living isolated cells and abnormal plants

a. *The absorption of fats.* The work of Czapek (20) and Schmidt (77) on the penetration of fine emulsified fats is frequently cited in support of the theory of *colloidal absorption* by living cells. A critical investigation to test this theory was recently undertaken by Rhine (74). Schmidt's technique was repeated. When filter paper saturated with various oils colored with Soudan III were inserted into longitudinal incisions in starved pea seedlings, Rhine found that both *free fatty acids and neutral oils* entered the cytoplasm from the intercellular spaces and appeared in the cytoplasm in the form of small droplets. From these experiments he concluded that the penetration was a physical matter depending in part upon the viscosity of the oil. When, however, the tests were made with *emulsions of linseed fatty acid in water*, no penetration into the cytoplasm took place. Further investigations to explain this discrepancy disclosed the fact that the penetration of the fats and oils into the cells was due to a water deficit in the walls of the starved etiolated pea seedlings, for when tests were conducted with normal seedlings well supplied with moisture in air and soil no penetration took place.

Moreover, comparing the respiratory quotients in the hypocotyls of fatty and starchy seeds for the purpose of determining the type of food used by the germinating pea seedling, Rhine rightly concluded that the form could not be fat, since the fatty seed hypocotyls were being furnished carbon in the same state of reduction as were those of the starchy seeds. Other experiments

showed that no diffusion concentration gradient of fats existed, nor were fats found "en route" in the cell walls in tissues through which formerly they were supposed to pass.

These critical investigations of Rhine appear to offer evidence that fat emulsions do not enter normal plant cells, but rigid experimental proof using animal cells has not been forthcoming.

Bechhold (4) is authority for the statement that fats are found in the truly soluble form (e.g., soaps) only at the time when they pass through the wall of the intestine, after which they immediately regain their colloidal condition. Unfortunately, he cites no experimental evidence. If his view is substantiated, the mechanism of penetration in such cases might be comparable with Witze-mann's conception (97) of the catalytic function of the leucoplasts in the condensation hydrolysis equilibrium involved in the formation and mobilization of starch and glycogen, the equilibrium being controlled by the second law of thermo-dynamics.

*b. The penetration of dyes.* Such an authority as Jacobs (41) states that many of the investigations on the penetration of acid and basic dyes (colloidal as well as non-colloidal) are worthless, partly on account of faulty technique and partly due to too broad generalizations from results on specific types of cells. Of the investigations relative to the conditions determining the penetrability of dyes, Ruhland's work (76) acquired prominence because his conclusions challenged Overton's view that "lipoid solubility" was the greatest factor determining ease of entrance into living cells. Ruhland demonstrated beyond doubt that not only the basic or acid nature of the dye but also, in the case of plant cells at least, the size of the molecule was the important factor. Colloidal dyes did not penetrate whereas non-colloidal dyes passed through. Höber (38) and more recently Gaedertz and Wittgenstein (23) have also confirmed Ruhland's conclusions (76).

However, it would be dangerous to infer that, because certain dyes having large molecules do not penetrate living cells, this is the determining factor in penetration. Beyond a certain limit this does not seem to hold true. This is exemplified by the results of permeability experiments with certain organic substances in which it has been shown that the size of the molecule may be of less importance than the presence of non-polar groups (51), for it is known that the introduction of such non-polar groups as  $-\text{CH}_3$ ,  $-\text{C}_2\text{H}_5$ , and  $-\text{C}_6\text{H}_5$  in place of hydrogen into the fatty acids increases their permeability (41).

#### C. CULTURE AND FIELD EXPERIMENTS WITH PLANTS

##### 1. *Distinction between permeability and absorption*

There is, in the literature, some confusion of the terms permeability and absorption. As Osterhout (66) has shown, the terms are not synonymous, because if ions are removed by reactions occurring in the cell or in the inter-cellular spaces, considerable absorption of the nutrient may take place,



although the true permeability of the substance might be quite low. Accordingly, with the same permeability, the amount of substances absorbed may vary greatly, depending on conditions. In the following discussion on the absorption of nutrients relative to the degree of dispersion this distinction should be borne in mind.

*2. The arguments advanced against the solution theory of plant nutrition*

Comber's stimulating paper (14) on the possible rôle of colloids in plant nutrition has attracted considerable attention. This authority rightly objects to "the tendency among many soil chemists to take so many things for granted without inquiring scientifically and experimentally into their truths" (17). He feels convinced that the assumption that material must be in solution in the soil before it can be of any use to the plant is quite unproved. In modification of present views, Comber postulates that the colloids of the soil play an important rôle by being directly absorbed by plants through the formation of a one phase system between the colloidal coating of the plant root hairs and the colloidal coating of the soil particles. Hence, according to this hypothesis, part of the nutrients absorbed on the soil surfaces are mechanically incorporated by the root hairs. Nutrients may in this way be absorbed without the necessity of going into solution. If this view is true it would follow that diffusion of salts in the soil, especially those of phosphorus and of iron, would not be an important factor in plant nutrition. It should be added that an alternative hypothesis to that of the direct absorption of colloids is also put forward by Comber (14), viz., that the organic excretions of the roots may exert a dissolution effect on the particles.

Clearly, this conception has an important bearing on soil solution studies and soil analyses, on which account rigid proof should be forthcoming before it is accepted. The arguments advanced in favor of the proposed modification will now be critically examined.

*a. The relation of the composition of the soil solution to the mineral elements taken up by plants.* It has been maintained on the basis of observations of Hall (33) and of Ramann (73) that, since the ratio of certain mineral elements assimilated by plants to the water transpired is greater than the ratio of these same elements to the water in which they are dissolved in the soil solution, therefore, the crop does not derive all its mineral matter by the simple flow of the soil water by osmosis into the roots. Accordingly, the examination of the soil solution to ascertain what soluble substances are present is no criterion of what a particular plant can get out of the soil.

This conclusion cannot be valid, for, as will be shown, the premise is unsound: (a) Based on a calculation of the amount of  $K_2O$  taken up by a clover crop to the water transpired, Hall claims that the concentration of  $K_2O$  thus obtained, viz., 0.006 per cent, is greater than in solutions obtained from the soil. That such a limit may not hold generally is shown by many analyses of the writer (84) of the soil solution obtained by displacement methods (52, 69)

from a large number of orchard soils in Pennsylvania in which the  $K_2O$  content was found to be 0.0078 to 0.0084 per cent. (b) Additional evidence is furnished from the carefully controlled and exhaustive experiments of Muenscher (61) and also of Prat (72) which provide rigid proof of the fact that transpiration does not play an important rôle in the supply of nutrients to the plants. They have shown conclusively that the quantity of an element absorbed by a plant bears no relation to the water absorbed and lost by transpiration. In other words, Muenscher and Prat have proved that plants can absorb water and solutes differentially, the quantity of an element absorbed being dependent upon growth as influenced by humidity and light intensity, rate of transpiration and concentration of solution. Hoagland (37), moreover, has shown that the rate of absorption is also dependent on the nature of the ions.

These conclusions have also been experimentally substantiated by Curtis' investigations (18). Transpiration, therefore, cannot have much direct influence on the movement of solutes. Chemical change in the cells is of far greater influence than the mere mechanical effects of transpiration (19).

b. *The absorption of silica by plants.* The experimentally established fact of the absorption of  $SiO_2$  from silica gels has also been produced as evidence in favor of the theory of colloidal penetration. The researches of Pfeffer (71), Gregoire (30), and especially of Jennings (42) leaves no room for doubt that silica when supplied either in the gel or sol condition is absorbed. Jennings used freshly prepared silica gels from sodium silicate, which were added to nutrient solutions in which wheat plants were grown. Analyses of these plants with the controls showed definite and relatively large absorption of silica.

But the conclusion that the absorption necessarily takes place in the colloidal condition *per se* is not valid. The facts, as we shall see, are also consistent with the solution theory.

(1) *The "solubility" of silica.* Truog (87), skeptical of the claim of the direct absorption of colloidal silica, offers as evidence against it that silica is soluble to the extent of 430 p.p.m. But difficulties at once arise with respect to the connotation "solubility." Lenher and Merrill (50) give 428 p.p.m. and Gile and Smith (27) give about 92 p.p.m. as the solubility of silica sols (silicic acid) in conductivity water. The former used S & S filter paper No. 589 and the latter Chamberlain filters. The researches of Joseph and Hancock (44) indicate a solubility of 32 p.p.m. for anhydrous silica.

All these investigators are careful to qualify their use of the term "solubility" by the explanation that all or part may be present as a finely dispersed sol rather than a true solution. It is, therefore, all the more interesting to note that more than twenty years ago Mylius and Grosehuff (63) by treating sodium silicate with hydrochloric acid prepared an unstable metasilicic acid that passed through a semi-permeable membrane.

Norton and Roth (64) also conclude that silicic acid may exist in true solution. The presence of definite silicic acids, such as orthosilicic acid ( $H_4SiO_4$ ),

in silica hydrogels and hydrosols, having the composition  $x\text{SiO}_2 \cdot y\text{H}_4\text{SiO}_4 \cdot z\text{H}_2\text{O}$ , in which the quantities of water and silica might vary from a true solution of silica in water through the highly dispersed gels, was indicated.

The writer (84) found that the silica in soil solutions obtained by displacement methods (69) dialyzed through collodion membranes prepared according to the method of Brown (7). McGeorge (57), too, found dialyzable forms of silica in all the Hawaiian soils examined and "little or no colloid forms." In the writer's experiments the dialysates contained 30 to 43 p.p.m.  $\text{SiO}_2$ . Moreover, owing to the well-known effect of the coagulating effect of ions, it is difficult to understand how, in the usual procedure for the analysis of clays, the sol could possibly remain stable in the presence of such large quantities of electrolytes, if the silica is not molecularly dispersed.

It is possible to interpret these results in accordance with the view presented by Hardy (34), viz., that silica may become soluble by the splitting off of small units of the adsorption complex,  $x\text{SiO}_2 \cdot y\text{H}_2\text{O}$ , owing to the ability of the "combined" water molecules to ionize.

The existence of pure silica hydrosols and hydrogels, as already mentioned, has been questioned. Hardy (34) concludes that the weight of evidence favors the view that hydrous silica is an ampholyte possessing an isoelectric point well on the acid side (pH 6.4), and records Williams' conclusions (95) that silica gel seems to possess a reticulate structure which when saturated contains water in two phases, the one absorbed at the surface of the material comprising the gel framework and the other filling the intermicellar spaces.

From the facts presented above, it would be unscientific to infer that because an increased absorption of silica by plants is found when silica gels are added to nutrient solutions absorption of the colloid takes place *per se*. The facts are obviously equally consistent with the simpler view of absorption from true solution.

(2) *The effect of silica gels on the absorption of other ions by plants.* Finally, it is of interest to inquire into the causes producing an increased absorption of phosphorus from rock phosphate in the presence of silica gels. Lemmermann and Weismann (49) attributed the increased yields to an increased assimilation of silica. This view was criticized by Gile and Smith (27), who contended that the beneficial action of silica gel on the growth of plants is to be attributed to the effect of silica on the solubility of rock phosphate. There is yet another possible explanation, viz., that derived from the application of the Gibbs-Donnan equilibrium law to the distribution of ions on the two sides of the semi-permeable membrane. This latter view is supported by the work of Butkewitsch and Butkewitsch (9), who have supplied experimental evidence on the influence of the presence of a non-diffusible ion (silica) upon the absorption of diffusible ions by plants. The application of the Gibbs-Donnan membrane equilibrium theory will be discussed in a subsequent paper.

c. *The absorption of iron by plants.* Comber (14) is unable to reconcile the absorption of iron by plants on calcareous soils with the solution theory.

This view is based on the insolubility of ferric oxide. It should be noted, however, that iron may exist in soils in comparatively large amounts in the *lower state of oxidation*. Thus, Vanstone (89) records the existence of iron in soil from the Folkstone Beds of England as the soluble and available ferrous phosphate.

Owing to the weakly basic character of  $\text{Fe}_2\text{O}_3$ , the ferric carbonate formed in calcareous soils is unstable, undergoing hydrolysis with formation of the sol  $x\text{Fe}_2\text{O}_3 \cdot y\text{H}_2\text{O}$ , or under some conditions, a precipitate of ferric hydrate (12). Hydrous ferric oxide is an amphoteric electrolyte with a pH probably of about 6.5. Its basic properties, however, outweigh its acidic properties and the "ferrites" so formed are quite unstable. Its acidic dissociation,  $\text{Fe}(\text{OH})_3 \rightarrow 3(\text{OH})^- + \text{Fe}^{3+}$ , therefore, would, as recorded by Hardy (34), be accompanied by the formation of the irreversible mono-hydrate. The precipitated hydroxide has a low solubility in water (1 p.p.m.); if, however, conditions permitted the rate of absorption even at this low concentration to be high, enough Fe might still be furnished for normal metabolism by the disturbance of equilibrium, as already discussed (p. 253). The investigations of Gile and Carrero (25), who showed that soils which yield iron sufficient for the growth of plants may not show a detectable amount of iron in the water extract, may be cited in support of this view. The experimental evidence, without exception, in culture solutions has shown that only traces of iron need be present in the solution for normal growth.

If, on the other hand, Joffe and McLean's conclusions (43) from laboratory experiments are applicable to soil conditions, then we must infer that iron cannot occur in molecularly dispersed solutions in normal soils and very little in the sol state, since no  $\text{Fe}(\text{OH})_3$  in molecularly dispersed solution was found by them higher than pH 3 to 4; and that even in the presence of  $\text{SO}_4$  ions no  $\text{Fe}(\text{OH})_3$  sol existed above pH 3.8. Apparently, however, in the presence of  $\text{NO}_3$  and Cl ions, the  $\text{Fe}(\text{OH})_3$  sol could exist as high as pH 5.4. These results indicate the importance of the consideration of the influence of the other ions in the soil.

Since the criterion of the sol condition used by Joffe and McLean was non-diffusibility through collodion sacs, this criterion, as was indicated in the earlier part of this paper, may invalidate the sharp line of distinction made by these authors between true solution and the sol and gel conditions.

It would be of interest to correlate Joffe and McLean's results with Gile and Carrero's culture experiments (26), in which these authors show that colloidal iron that was not permeable to a parchment membrane was available to plants in an acid medium and to a limited extent in a neutral medium but not when  $\text{CaCO}_3$  was added. Unfortunately, Gile and Carrero do not give the pH of their solutions.

In an earlier paper (25) Gile and Carrero, comparing the availability of dialyzed iron with ferric chloride to rice plants in culture solutions, conclude that rice could not assimilate "colloidal iron" but that the plants absorbed the

fraction of the iron that was molecularly dispersed from the dialyzed ferric oxide sol.

Hardy (35) believes that complex coördinated metal anions may readily penetrate and suffer translocation in the plant. This belief is founded on the results obtained by many investigators who have used different iron compounds in nutrient culture experiments; but, as there is no definite information on the composition of these complex organo-metallic compounds (36), the assumption that they are absorbed by plants seems unwarranted. There can be little doubt that the complex ferro-citrates and ferro-tartrates, as obtained by extracting iron oxides with citric and tartaric acids, are colloidal and that on standing they gradually undergo hydrolysis and precipitate out; but there is still some ferric ion in solution even after 60 days (36). In this connection it is interesting to note that analysis by the writer of a sample of Merck's "dialyzed iron containing 5 per cent  $\text{Fe}_2\text{O}_3$ " gave, after coagulating with  $\text{MgCl}_2$  and filtering, 0.5 per cent of chlorine. Tested with potassium sulphocyanate the filtrate gave a slight blood red color. The presence, therefore, of some Fe in solution is indicated. It follows that the colloid-solution equilibrium hypothesis advanced earlier in this paper (p. 253) may also be applied to explain the penetration of iron (and aluminum) into cells. According to this view we would have:



Relative to the absorption of other ferric sols by plants the experiments of Parker (70) show that colloidal ferric phosphate hydrosol, that was non-diffusible through collodion sacs, was not available to plants.

Field experiments on the subject show strange contradictions. Thus, Gile and Carrero's field experiments on calcareous soils indicate that iron-induced chlorosis may result in plants growing upon them. We are, therefore, on the horns of a dilemma. Iron is available to plants on the chalk soils of England, but in the calcareous soils of Hawaii it is not available in sufficient amounts to prevent chlorosis. The results on the Hawaiian soils do not support the explanation offered (14, 35) for the absorption of iron, viz., the formation of *organo-compounds* with such substances as sugars, starches, certain proteins, etc.

The conclusion seems inevitable, therefore, that more facts are needed before any claim to the absorption of colloiddally dispersed iron by plants can be made. All the facts can readily be explained on the basis of the iron of soils being absorbed in the molecularly dispersed state.

*d. The availability of mineral phosphates.* If the phosphorus applied both in the "raw" mineral form and as superphosphate is present in the colloiddally dispersed phase, it would not be possible, as many investigators have done, to find a correlation between the amount of water-soluble phosphorus, the amount of phosphate applied and the response of crops. In what follows, we shall discuss some of the salient points with reference to the conditions rendering the phosphorus of mineral phosphates available.

(1) *Superphosphates*. In a very ingenious discussion of the availability of phosphorus, Murray (62) points out that the compounds into which the soluble phosphates are converted by reversion in the soil will consist of particles of different sizes; some, therefore, will have larger and some smaller *surfaces*. As the particles below the critical stage (circ.  $200\mu\mu$ ) representing the transition to the colloidal state will undergo comparatively rapid solution, it follows that the rate of absorption of ions by the plant will be determined by the number of particles below the critical stage.

Murray cites the law of diminishing returns, which Mitscherlich and Baule (60) have attempted to express as a fundamental law, in support of this view. Thus, when the particles below the critical stage are formed in such large numbers that equilibrium exists between the rate of absorption by the plant and the rate of solution, no appreciable difference in the yield would be expected to occur by the addition of more fertilizers. This is what happens in most cases, although exceptions to the general rule are known (48).

Murray's views on the formation of solid particles are based on the hydrolysis hypothesis. The investigations of Cameron and Bell (10) lead to the conclusion that the addition of mono-calcium phosphate to the soil results in the formation of the secondary salt  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ .



They concluded that no definite compound existed intermediate between  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Ca}(\text{OH})_2$ , but only a series of solid solutions. Austin's (1) results on the electrometric titration of  $\text{Ca}(\text{OH})_2$  and of  $\text{CaCO}_3$  with mono-calcium phosphate are essentially in accord with the views of Cameron and Bell. Both Warrington's (91) and Bassett's (2) investigations, however, indicate that only the tricalcic phosphate  $\text{Ca}_3(\text{PO}_4)_2$  and the hydroxy-apatite  $\text{Ca}_5(\text{PO}_4)_3 \cdot \text{Ca}(\text{OH})_2$  can be in stable equilibrium with an aqueous solution, and that the latter is the only calcium phosphate that can permanently exist under normal soil conditions. Which of the foregoing conclusions apply to the actual conditions in the soil, it is impossible to say. All that is certain is that calcium mono-phosphate is converted into a substance (or substances) that is very much less soluble than the original substance and that, possibly, the conversion may be into very insoluble forms. Murray's deductions are, therefore, in no way irrational. But the actual conditions existing in the soil may not be capable of explanation wholly by solution forces, as he supposes; for, apart from the possibility of the formation of aluminum and iron phosphates in acid soils, there are unquestionably other forces operative, in addition to those pointed out on page 263, to disturb the equilibrium conditions, such as those due to absorption of the  $\text{PO}_4$  ions by the soil colloidal complexes. The tenacity with which these absorbed *ions* will be held will vary with the potential differences developed and, therefore, upon other cations and anions present in the absorption complex (16, 79, 81). The concentration of the  $\text{PO}_4$  on the colloidal surfaces will be determined by the Gibbs-Donnan equilibrium law.



The experimental work in support of these facts is considerable, of which some only will be cited: (a) The  $\text{PO}_4$  ions of superphosphate may be so strongly absorbed by some soils that acids may not dissolve more phosphorus from such soils treated with superphosphates than from soils supplied with rock phosphate (98). (b) In field experiments it frequently happens that as much as 20 per cent of the applied phosphorus is not recovered by the crop (27). Wheeler and Adams (93) concluded from their experiments on peat and muck soils that "saturation" of the soil colloids was necessary before additions of phosphates became effective.

Gordon and Starkey (28, 80), working with artificial iron and aluminum gels, have shown, as would be expected from theoretical considerations, that the greater the hydrogen-ion concentration of the surrounding solution, the lower the absorption of  $\text{PO}_4$  ions and vice versa. Nevertheless, leaching of phosphorus may occur. A slight loss of phosphorus from a loam soil derived from Triassic Red Shale has been noted in the New Jersey Station cylinder experiments (5), especially in the larger phosphatic applications. On some North Wales shaly loams and even in some heavy clay loams Robinson and Jones (75) found considerable leaching of phosphorus—applied as slag—into levels below 18 inches, in a region where the leaching of the soil by percolating waters is above normal.

The foregoing evidence on superphosphate may be insufficient to provide rigid proof that the facts are best in harmony with the solution theory; but, on the other hand, no experimental work on superphosphates has, thus far, furnished any support for the theory of the absorption of colloids. If rigid proof were forthcoming that silica gels or sols are directly absorbed by plants, then, since it forms the greater portion of the outer coating of the soil colloidal particles, phosphorus and other ions absorbed by this hydrogel might be conceived to be carried into the root hairs with the silica, but until additional facts are obtained the view must be held to be purely speculative.

(2) *Sparingly soluble phosphates.* Is it valid to deduce from the fact that fertilizers, like basic slags and mineral phosphates, are "sparingly" soluble that solubility is not the *dominating* condition of availability, as Comber (14) suggests? The terms "sparingly soluble" and "valuable" applied to such fertilizers are purely relative. On some soils and for some plants, especially the *solanaceae*, as will be discussed in a subsequent paper, the mineral phosphates may be quite unavailable. Under favorable conditions, however, in some soils the mineral phosphates, especially "floats" (raw rock phosphate ground finer than 200 mesh), may be as available as the superphosphates.

(a) *Solubility and availability of "floats."* Due to their variable content of  $\text{CaCO}_3$  there is considerable variation in the solubility of the natural rock phosphates in water, carbonic and citric acids, etc. The extensive bibliography on the subject is recorded by Cameron and Hurst (11). These latter investigators arrived at the conclusion that rock phosphate undergoes hydrolysis as follows:



The "solvation" of this mineral by water is, therefore, a solution not of the tertiary phosphate but of its decomposition products. It is then these secondary products that are subjected to the action of the solvent forces of the soil solution. Moreover, the solubility of these products is related to their availability. Thus, Vanstone (88) has found that all the phosphates of calcium examined by him were practically completely soluble in 2 per cent oxalic acid in half an hour at ordinary temperatures and that the intake of phosphorus by the bean plant is proportional to the oxalic acid solubility.

(b) *Acids as a factor.* There is some evidence to indicate that acids, especially carbon dioxide, resulting from the decomposition of organic matter may, under some soil conditions, be an important factor. The laboratory experiments of Truog (86) and the field and laboratory work of Breazeale and Burgess (6) may be cited in this connection. The latter investigators found that in the case of wheat plants the relative absorption of  $\text{PO}_4$  from rock phosphate in soil culture solutions, treated with "floats" contained in distilled water only, was only 0.0104 gm.  $\text{PO}_4$ , but 0.0188 gm.  $\text{PO}_4$  when the solutions were saturated with  $\text{CO}_2$  and 0.0206 gm.  $\text{PO}_4$  from a dilute aqueous solution of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . The availability of the rock phosphate used by these investigators in soil saturated with  $\text{CO}_2$ , therefore, is not much less than that of superphosphate. Although, as will be shown in a later paper, it is unlikely that the soil solution is saturated with  $\text{CO}_2$ , nevertheless, barring secondary reactions, the marked influence of this solvent on the availability of "floats" is indicated. It is probable that the reason why the earlier investigators failed to determine the influence of the  $\text{CO}_2$  factor on the *availability* of rock phosphate was due not only to the reasons cited by Truog (86) and to reasons mentioned later (p. 263), but also to the unfortunate selection of the solvent used in extracting the phosphoric acid. When the extraction is carried out by means of the acids (citric, nitric) used by the earlier investigators, a reverse reaction sets in between the soluble calcium salt formed and the phosphoric acid that has become soluble, resulting in the precipitation of the phosphate (88).

From the foregoing it follows that an exact formula for the solubility of rock phosphate is not possible. It may be signified by the expression  $s = \int \frac{\text{H}^+}{\text{Ca}^{++}}$ , indicating that the solubility increases with the hydrogen-ion concentration of the solution. We should expect, therefore, as indeed Gedroiz (24) and McCall (56) and others have already shown, an increased availability of these naturally occurring phosphates on unsaturated soils. It has already been mentioned that Vanstone (88) found that the solubility of rock phosphate in 2 per cent oxalic acid corresponded with the rate at which mineral phosphates are absorbed by plants and, furthermore, Kelly's (47) laboratory experiments have indicated a depression in the solubility of  $\text{Ca}_3(\text{PO}_4)_2$  in the presence of  $\text{CaCO}_3$ .

However, the solution of the problem of the availability of rock phosphates

to plants is not so simple as might be inferred from solubility results. The favorable factors leading to the solution of rock phosphate in the soil may be opposed by secondary reactions that tend to the "fixation" by bacteria (85) and to the precipitation by the hydrolyzing soil exchange bases, e.g., Al, Fe, (79) of the phosphorus thus rendered soluble. The new series of fertilizer plot experiments of the Pennsylvania Experiment Station, which were commenced in 1920, may be cited in illustration. In these experiments the additions of superphosphate and rock phosphate, respectively, to the *lime with manure* plots (pH 6.8) have given only approximately 15 per cent higher yields of corn, oats, and wheat than on the *lime without manure* plots (pH 5.9 to 6.0).

Current explanations, therefore, relative to the results obtained on the neutral or slightly acid soils of some of the eastern and middle western experiment stations, supplied with sufficient rainfall, to the effect that the amount of decomposing organic matter is the most important factor conditioning availability of rock phosphate, require qualification. Generalization of the explanations presented by Breazeale and Burgess (6) of their results obtained on the Western alkaline soils which they maintain contain no free  $\text{CO}_2$  and which give no response to rock phosphate additions is, therefore, limited by other factors.

In many cases conclusions as to the relative availability of rock phosphate (and superphosphate) have been based on growth in sand culture experiments using mineral nutrient solutions. Such experiments, obviously, do not show the relative availabilities under field conditions.

There is evidence that the mineral acids and possibly also acetic, butyric and lactic acids formed in the soil increase the availability of sparingly soluble phosphates. This is supported by the work of Hopkins and Whiting (40) on the availability of rock phosphates in the presence of ammonium salts, and also by the Lipman process (53, 54) of rendering floats available by utilizing the oxidation of sulfur by micro-organisms.

(c) *Absorption of  $\text{PO}_4$  from very low concentrations.* The concentration of phosphate ions in all soils is low. Numerous investigators, working with culture solutions, have shown that plants can develop normally in very dilute solutions of  $\text{PO}_4$ . Thus, Breazeale and Burgess (6) found that oat and barley seedlings can absorb  $\text{PO}_4$  sufficient for normal development in a nutrient solution containing only 0.50 p.p.m.; and Parker (70) found that corn will make maximum growth in culture solution containing only 0.25 p.p.m.  $\text{PO}_4$ . If these results are applicable to field conditions, the small solubility of rock phosphate would present no difficulties to the acceptance of the older solution theories.

Comber (15) points out that the naturally occurring phosphates do not seem to possess colloidal surfaces. But even if such a phosphate as "floats" was prepared in the colloidal condition and applied to the soil, it is questionable if carbon dioxide and the other solvents present would react with the colloid, owing to the influence of the electric charge carried by it.

The evidence presented affords no indication that the availability of rock phosphates is determined by any colloidal behavior. Rather does it point toward the conclusion that its availability is a question of absorption from solution as determined by the laws of diffusion and mass action, through the operation of which the rate of absorption of  $\text{PO}_4$  ions by the plant is a function of the rate of solution of the mineral phosphate.

The behavior of other minerals is similar. Thus, the potassium or orthoclase has a solubility of 282 p.p.m. in distilled water. One could hardly, therefore, attribute the results obtained by Haley (32), in which 200-mesh orthoclase used in sand cultures furnished potassium at a rate ample to produce large yields of buckwheat, to the colloidal condition of the orthoclase.

(d) *Iron and aluminum phosphates.* Although there is evidence (58) to indicate that the *artificially prepared* phosphatic salts of iron and aluminum are more available to plants than the *naturally occurring* phosphates of these metals, there is nothing to indicate that colloidal behavior is the cause of this difference. Unfortunately, Marais (58) does not give the source of his "C.P." materials, nor the relative fineness of division of any of the phosphates used. Although it is possible that the greater fineness of division of the artificial phosphates as well as the tendency towards increasing basicity and consequent insolubility of the natural products, as weathering proceeds, may be contributory causes, other factors may predominate. Thus, in the case of artificially prepared ferric phosphate, for example, Cameron and Bell (10) have shown that the acidic properties of the salt prepared by precipitation may be very pronounced, even after 100 washings, due to absorbed  $\text{PO}_4$  ions. The salt thus prepared is more soluble and, therefore, would be more available than the pure product.

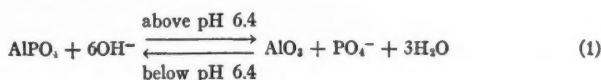
Moreover, since Marais (58) did not find the *mineral* aluminum and iron phosphates of any value except in the presence of ammonium compounds, it would be logical to infer that the availability of these mineral phosphates in the presence of nitrogenous salts would be due to the action of the nitric acid formed (40), the rate of solution being determined by the number of particles below the critical stage (p. 260). However, it must be admitted that our knowledge of the conditions rendering the various phosphates in the soil soluble are very unsatisfactory. Over twenty years ago it was suggested by Whitson and Stoddart (94) that the reason why acid soils act as if deficient in phosphorus is due to the formation of aluminum (and iron) salts. Vanstone's (89) experiments on the solubility of soils in citric acid give confirmative evidence of the relative insolubility of the phosphorus of acid soils. But the findings of some investigators (58, 93) that the availability and, therefore, presumably the solubility of aluminum phosphate is increased by liming is not explicable on theoretical grounds. The equilibrium conditions of a solution involving Al (or Fe), Ca,  $\text{PO}_4$ , H and OH ions may be written



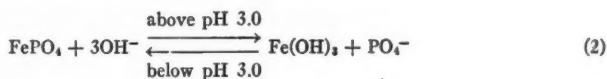


As the solubility product of  $\text{AlPO}_4$  is less than  $\text{Ca}_3(\text{PO}_4)_2$ , most of the  $\text{PO}_4$  ions, at pH 7.0 and above, would be precipitated as  $\text{AlPO}_4$  (or  $\text{FePO}_4$ ). The tendency of lime would, therefore, be to depress the solubility of aluminum and ferric phosphates.

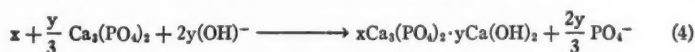
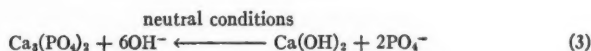
Teakle (81) suggests from the results of his experiments that the following reactions may take place.



and



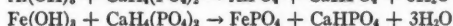
also



alkaline conditions

Under more alkaline conditions the calcic phosphate would give rise to a basic phosphate and  $\text{PO}_4$  ions. But considerations of the solubility products of these substances lead to difficulties in accepting Teakle's conclusions *in toto*.

Austin (1) has challenged the belief that the formation of insoluble complexes of iron and aluminum phosphate in acid soils is the cause of the low availability of phosphorus found in many acid soils. He did not, however, as the title would seem to suggest, use any soils in his experiments. The data was obtained by working with the pure hydrosol or hydrogel bases assumed to be present in soils, by titrating electrometrically with  $\text{CaH}_4(\text{PO}_4)_2$ . Austin's conclusion is based on the fact that the titration of  $\text{Al}(\text{OH})_3$  and  $\text{Fe}(\text{OH})_3$  with  $\text{CaH}_4(\text{PO}_4)_2$  resulted in 82 per cent and 40 per cent, respectively, of the phosphorus still being in solution at pH 5.4, from which fact he concludes that at pH 5.4 the equations



do not go to completion. In other words, in the presence of Al (or Fe), Ca,  $\text{PO}_4$ , H and OH ions a fairly acid soil would not have all the  $\text{PO}_4$  ions combined with Al (or Fe).

The actual conditions in the soil are undoubtedly complex. Hydrous alumina has an isoelectric point of 6.5. If we consider its acidic dissociation

$\text{Al}(\text{OH})_3 \rightarrow \text{Al}^{+++} + 3\text{OH}^-$  obviously the addition of an acid will result in the formation of water by the combination of the  $\text{H}^+$  of the acid and  $\text{OH}^-$  of the alumina. It follows, as Hardy (34) has indicated, that the colloidal hydrous alumina would be removed further from its isoelectric point and the potential difference between its surface and the dispersion medium would be increased. The addition of an anion, such as  $\text{PO}_4$  or  $\text{SiO}_2$ , would—depending on the other anions and cations present—result in the formation of insoluble complexes. The precipitating power of other anions would depend on their relative electro-negative affinity. As the soil solution became more acid we should expect Al (and Fe) ions to come into solution, for both aluminum phosphate and ferric phosphate become more soluble the higher the hydrogen-ion concentration of the medium. The system resulting would depend on the solubility products of the substances formed.

(e) *Basic slags.* The expression “insoluble but available to plants” by which clays have been characterized does not, without qualification, appear justifiable. The old Thomas slag obtained by the Thomas-Gilchrist-Bessemer process, which was produced by the addition of a basic “liming” of  $\text{CaCO}_3$  and  $\text{MgCO}_3$ , was of a fairly constant composition, the phosphorus of which is from 80 to 95 per cent available to plants and showed close correlation with solubility in 2 per cent citric acid. The first “run off” used for the agricultural slag has a high phosphorus content. This Gilchrist-Bessemer process, however, has now, for economical reasons, been largely superseded in Europe by the Siemens-Martin process, in which a calcium fluoride flux is used, resulting in slags of very variable composition and phosphorus availability (6 to 50 per cent), but which show little correlation with solubility in 2 per cent citric acid. The slags from the acid-Bessemer and Basic Open Hearth processes used in the United States are not high in phosphorus and, moreover, the ores used for the making of steel in this country are invariably low in phosphorus.

Stead, as recorded by Vanstone (90), gives  $\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaO} \cdot \text{CaSiO}_3$  as the composition of the low acid soluble slags. The most soluble phosphate has the composition  $5\text{CaO} \cdot \text{SiO}_2 \cdot \text{P}_2\text{O}_5$ . When these silico-phosphates are applied to the soil the combined CaO is freed as weathering proceeds, permitting the ready dissolution of the  $\text{P}_2\text{O}_5$  radical. The period of time for this weathering process to occur and, therefore, the availability varies with the nature of the slag. The smaller the amount of silicate in the slag the higher the availability.

The behavior of slags in the soil, therefore, does not tend, without further experimental evidence, to support any theory of colloidal absorption by plants.

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## CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: III. CHEMICAL STUDIES OF TWO FLORIDA PEAT PROFILES

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In a previous contribution (10), the authors suggested a system of proximate analysis of peat material which accounts for about 90 per cent of its constituents in the form of definite chemical complexes. This system of analysis has been devised with the idea of throwing light upon the chemistry of the organic constituents of peat and upon the chemical processes involved in their formation from the natural plant materials. It was found that in all the lowmoor and sedimentary peats examined, the celluloses are completely or almost completely decomposed while some of the hemicelluloses, largely of a hexosan nature, are left; the highmoor peats, however, were found to contain considerable quantities of both celluloses and hemicelluloses. This marked difference in the chemical composition of these types of peat was found to be due not only to the specific environmental conditions prevailing in the peat bogs during plant growth and decomposition, but largely to the chemical nature of the organic constituents of those plant associations which gave origin to the specific peat formations.

Lowmoor and sedimentary peats are also known to contain large quantities of organic nitrogenous complexes, the nitrogen content of these specific types of peat being considerably higher than that of the plants from which they have originated; on the other hand, the highmoor peats contain only a limited amount of organic nitrogen compounds, frequently even less than the plants from which these peats have originated. These important differences were explained as due largely to the nature of the decomposition processes of the various plant constituents, brought about by the microorganisms which are active in the formation of the different types of peat. The highmoor peats contain a considerably larger amount of fatty and waxy substances than the lowmoor and sedimentary peats. The low mineral content and high acidity of the highmoor peats, the fairly high mineral content and limited acidity of the lowmoor peats, and the great abundance to almost predominance of mineral matter and neutrality to alkalinity of the sedimentary peats are also characteristic phenomena for differentiating the chemical composition of these important formations of peat.

The following studies deal with two more profiles, one lowmoor and one sedimentary, which were taken from a distinctly different region and which result from different plant associations and are formed under conditions

entirely different from those of the New Jersey peat profiles. These studies were undertaken with the idea of throwing further light upon the chemical composition of peat and upon the chemical and biological processes involved in its formation from natural vegetation.

The lowmoor peat used in these investigations represents a typical profile of the Everglade peat area in Florida. The authors are indebted to Dr. R. V. Allison of the Everglade substation at Belle Glade, for supplying the necessary material. The profile was taken about four miles south of Lake Okeechobee and about a quarter mile west of Okeelanta.

The sedimentary profile was taken from a typical allochthonous peat formation, referred to by European investigators as Gytjtja. This peat has been formed in standing water, in a lake known as "Mud Lake," about 20 miles northeast of Ocala in Northern Florida and about 80 miles SSW of Jacksonville, Florida. The lake is fed by a series of springs and is bordered on the southwest and north by swamps, and on the east by a wooded hill; it drains on the northwest side into a river by means of a small creek. At the time of sampling, the surface of the peat was about three feet below the surface of the lake, while its depth extends to about forty-five feet. The authors are indebted to the Cummer Lumber Co. for supplying the required material from this peat formation.

The Everglade lowmoor, or saw grass, profile was divided into seven sections, designated as follows:

Number of sample	Depth cm.	
50	0-7.5	Dark brown to black, finely decomposed surface material with varying quantities of undecomposed fiber
51	7.5-30	Upper fibrous layer ( <i>Cladium</i> ) gradually grading into pure <i>Cladium</i> peat
52	30-45	Layer of dense fibrous peat derived entirely from saw grass ( <i>Cladium</i> )
53	45-55	Lower depth of fibrous layer and adjoining black compact layer of sedimentary peat
54	55-70	Central portion of sedimentary layer consisting of black, compact, colloidal material, with a minimum of fibrous material
55	75-120	Lower portion of sedimentary layer with adjoining upper portion of lower fibrous layer
56	125-135	Lower fibrous layer of <i>Cladium</i> peat

At a depth of 185 cm. rock formation is underlying the peat area.

The fibrous layer consists of practically pure saw grass or *Cladium*. The sedimentary or plastic layer is a typical allochthonous formation resulting from an accumulation of wind blown, sedimentary vegetable material as well as of various water plants formed in permanent quiet bodies of water. When the lake-formed deposits have been built up to a point less than 10 feet below the average water level, species of *Nymphaeaceae*, *Cladium*, *Sagittaria*, etc., appear which gradually give rise to the brown fibrous peat which is autochthonous in nature. This peat is later invaded by lowland trees. According to Forsaith (4), the allochthonous peat contains calcareous remains of *Chara*,



diatomaceous tests, limy silts, etc.; pollen grains of Abietineae and catkin-bearing angiosperms, spores of ferns, fungi; strips of cutinized epidermis, idioblasts of water lilies and fragments of woody and herbaceous plants; animal derivatives, such as chitinated portions of insects, spicules of fresh water fungi, infusoria, shells of mollusks and protozoa. This mass of material can be distinguished even from the well-decomposed plant residues of autochthonous peat (when the water level was low), both by microscopic structure and by chemical composition.

Dachnowski (3) analyzed both the sedge peat and the sedimentary peat in approximately the same part of the Everglades from which the samples used in these investigations were obtained, with the following results: The air-dry sedge peat contained 11.24 per cent water, 7.2 per cent ash, 19.63 per cent protein and 0.25 per cent fat, whereas the sedimentary layer contained 9.81 per cent water, 32.67 per cent ash 15.75 per cent protein and 0.15 per cent fat. On an ash-free and moisture-free basis, the sedge peat contained 24.07 per cent protein and the sedimentary peat 27.38 per cent.

Miller (6) reported the following inorganic constituents in the plants of *Cladium effusum*, on per cent basis of dry material:  $\text{SiO}_2$  - 0.30;  $\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$  - 0.11,  $\text{CaO}$  - 0.57,  $\text{MgO}$  - 0.11,  $\text{Na}_2\text{O}$  - 0.16,  $\text{K}_2\text{O}$  - 0.35,  $\text{P}_2\text{O}_5$  - 0.07, and nitrogen - 0.8. The saw grass peat contained, on a similar basis: total ash 6.8 to 7.7,  $\text{SiO}_2$  - 1.95 to 2.04,  $\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$  - 0.60 to 0.69,  $\text{CaO}$  - 2.74 to 3.02,  $\text{MgO}$  - 0.44,  $\text{Na}_2\text{O}$  - 0.14 to 0.19,  $\text{K}_2\text{O}$  - 0.06 to 0.11,  $\text{P}_2\text{O}_5$  - 0.13 to 0.15, nitrogen - 3.32 to 3.84 per cent. The results show that with the decomposition of the saw grass, there is a considerable loss of potassium and a gain in nitrogen and various bases. Assuming that there was no loss of silicon in the process of peat formation from the saw-grass plants, Miller calculated that about seven parts of plant material were required to produce one part of peat.

Rose (7) found 0.067 per cent  $\text{K}_2\text{O}$  in the saw grass peat and 0.122 per cent in the sedimentary peat. Hammar (5) made a detailed analysis of the inorganic constituents of several of the peat areas in the Everglades, namely the saw grass peat, the sedimentary peat itself which gives the so-called custard-apple soil or plastic soil, and an intermediary peat, which gives the so-called elderberry soil. His results can be summarized as follows, on a per cent basis of dry peat:

CONSTITUENTS	SAW-GRASS PEAT	SEDIMENTARY PEAT	INTERMEDIATE PEAT
	per cent	per cent	per cent
Organic matter.....	86.37	44.52	77.65
Nitrogen.....	2.79	1.47	2.65
Ash.....	13.63	55.48	20.35
$\text{SiO}_2$ .....	4.12	35.81	9.87
$\text{Fe}_2\text{O}_3$ .....	1.01	5.04	1.81
$\text{CaO}$ .....	5.21	3.81	5.25
$\text{MgO}$ .....	0.10	0.09	0.13
$\text{P}_2\text{O}_5$ .....	0.42	0.48	0.42
$\text{Al}_2\text{O}_3$ .....	0.30	5.08	0.78

The sedimentary peat profile was taken, in one foot sections, from the surface of the peat (3 feet below surface of lake) to a depth of 12 feet. These twelve sections were combined so as to give only 6 layers of the profile, including the upper one foot, 1-2, 2-4, 4-6, 6-8, 8-10, and 10-12 feet. In the analyses reported in the previous papers (11), only sedimentary layers were analyzed from lowmoor and highmoor peat profiles. Those layers were characterized by a high ash content, frequently as much as 60 per cent of the total dry material, a high protein content (10 to 24 per cent), practically no cellulose, varying amounts of hemicellulose and a low ether soluble fraction.

The chemical composition of the Everglade peat has attracted considerable attention, just as the whole problem of the utilization of the Everglades

TABLE 1  
*Chemical composition of Florida (Everglade) peat profile*  
On per cent basis of dry material

NUMBER OF SAMPLE	PEAT FORMATION	DEPTH OF LAYER	ASH	CRUDE PROTEIN	ETHER SOLUBLE	COLD AND HOT WATER SOLUBLE	ALCOHOL SOLUBLE	HEMICELLULOSE	CELLULOSE	LIGNIN	TOTAL
		cm.									
50	Surface layer	0-6.5	12.09	22.75	2.96	1.31	1.22	6.87	0.30	43.72	91.22
51	Upper fibrous layer	6.5-26	10.00	23.06	2.98	1.73	1.06	6.41	0.28	46.12	91.64
52	Typical saw-grass peat	26-40	6.86	22.25	3.16	1.60	1.31	8.04	0.43	44.90	88.55
53	Lower fibrous layer	40-50	8.05	21.25	3.21	1.45	1.39	7.63	0.43	47.63	91.04
54	Sedimentary layer	50-62	59.60	9.00	2.97	1.07	0.45	2.19	0	19.33	94.61
55	Lower portion of sedimentary layer	62-70	42.37	12.98	1.46	0.82	0.68	2.64	0	28.47	89.42
56	Lower fibrous layer	110-120	15.14	20.38	1.58	0.84	1.23	4.30	0	48.44	91.91

However, as seen from the previous references, very little attention has been paid to the chemical nature of the organic complexes in this most extensive peat formation, and to the influence that this may have upon the productivity of the peat when it is changed by draining and cultivation, into a crop-producing soil.

The seven horizons of the saw-grass peat profile were analyzed according to the method previously described. The results are presented in table 1. To enable us to compare the abundance of the various organic complexes in the several layers of the profile, the figures representing the hemicellulose, lignin and protein content of the different horizons are calculated on an ash-free, dry basis and reported in table 2.

The results of the chemical composition of this lowmoor peat profile confirm

the observations of other investigators in regard to the organic matter, ash and nitrogen content of the saw-grass and sedimentary peat formations. Not only the younger saw-grass peat or the layers above the sedimentary peat but also the older saw-grass peat, or the layer below the sedimentary peat contain about 93 to 85 per cent organic matter, while the sedimentary material itself is very high in inorganic matter and contains only 40.40 to 57.43 per cent of organic substances.

The upper horizons of the profile, namely the younger saw-grass peat, still contain small amounts of cellulose, while the sedimentary peat and the layers of older saw-grass peat are entirely free from cellulose. The hemicellulose content is also considerably higher in the younger saw-grass peat than in the sedimentary and the older fibrous peats. One must recall in this connection that the saw-grass plants were found to contain 28.3 per cent cellulose in the stems and 30.7 per cent in the roots, as well as 21.5 and 20.8 per cent pentosan in the stems and roots respectively. In other words, as a result

TABLE 2  
*Chemical composition of the organic matter of an Everglade peat profile*  
On per cent basis of moisture-free and ash-free material

NUMBER OF SAMPLE	HEMICELLULOSE	LIGNIN-LIKE COMPLEXES	PROTEIN
50	7.81	49.68	25.85
51	7.12	51.24	25.62
52	8.64	48.23	23.90
53	8.29	51.66	24.18
54	5.42	47.80	22.28
55	4.58	49.43	22.53
56	5.06	57.05	24.01

of decomposition of the plant residues by microorganisms in the process of peat formation, nearly all the cellulose and a large part of the hemicellulose have disappeared. It is again important to call attention to the fact pointed out previously (11) that while in the fresh saw-grass plants most of the hemicelluloses are made up of pentosans, in the peat itself most of the hemicellulose is of a hexosan nature. This points to as nearly complete a decomposition of the pentosans as of the celluloses and to an accumulation of certain hemicelluloses of a hexosan nature resistant to decomposition, some of which are of plant origin and some have no doubt been synthesized by the microorganisms bringing about the decomposition processes in the peat.

The protein content, determined by total nitrogen, is considerably higher in the saw grass than in the sedimentary peat, but the difference becomes less marked when calculated upon an ash-free basis. The same is true of the lignin-like complexes, or that fraction of the organic matter which is insoluble in ether, alcohol, boiling dilute hydrochloric acid and cold 80 per cent sulfuric acid, followed by boiling in 5 per cent of this acid. The lower or

older saw grass peat contains considerably more lignin and less of the ether and water soluble materials as well as of the hemicellulose fraction than the younger saw-grass layers of the peat profile. This, as well as the complete absence of cellulose, indicates that considerably greater decomposition has taken place in the older saw-grass peat than in the younger formations.

Definite chemical differences are thus found to exist not only between the saw-grass and sedimentary peats, but also between the younger and older saw-grass peat formations. It would be of considerable interest to determine whether these differences have an influence upon the rapidity of decomposition of peat and the liberation of the nutrients, especially of the nitrogen, in an available form. Fortunately, the sedimentary peat forms large outcroppings, where no surface layer of saw-grass peat has as yet been formed, namely in the form of a narrow zone just adjoining the lake Okeechobee. When drained and cultivated, the sedimentary peat, known locally as "custard-

TABLE 3  
*Evolution of CO<sub>2</sub> and formation of nitrate from 100-gm. portions of different layers of an Everglade peat profile*

NUMBER OF LAYER	TOTAL CO <sub>2</sub> -EVOLUTION (MGM. OF C)			NITRATE FORMATION (MGM. N)	MOISTURE CONTENT (PER CENT)
	9 days incubation	18 days incubation	32 days incubation	32 days incubation	
50	15.0	24.7	41.8	13.24	66.8
52	8.3	13.5	24.9	12.5	71.4
53	7.0	10.8	18.2	5.5	88.3
54	2.5	4.0	6.5	5.1	85.0
55	3.6	6.3	12.0	1.6	75.8
56	3.8	7.4	12.9	0.6	83.4

apple soil," is found to be very fertile, while the saw-grass peat is of considerably lower fertility as far as the growth of cultivated plants is concerned.

Although a detailed survey of the microorganisms found in different layers of various peat profiles and of the microbiological processes taking place in the formation of peat and in the decomposition of peat when the latter is drained and cultivated, is reserved for a later publication, certain data will be presented here in an attempt to throw light upon any possible differences in the transformations which take place in the saw-grass and sedimentary peat regions in the Everglades when they are subject to the action of a mixed flora and fauna of microorganisms.

One-hundred-gram portions of the natural moist peat were taken from the various layers of the profile, placed in aeration flasks and incubated for 32 days, at 25°C. At the end of that period of time the total nitrate nitrogen was determined. The evolution of carbon dioxide from each 100-gram portion of moist peat and the liberation of nitrogen in the form of nitrate are reported in table 3.

The results show that there is a gradual decrease in the rapidity of liberation of carbon dioxide and formation of nitrate nitrogen with the depth of the peat profile. One must consider of course the fact that the moisture content of the material taken from the different layers varies, and, when calculated on the basis of actual amount of dry peat undergoing decomposition, different results will be obtained: 33.2 gm. of the dry peat of No. 50, or the uppermost layer, gave off, in 32 days, 41.8 mgm. of C as  $\text{CO}_2$  and allowed the accumulation of 13.2 mgm. of nitrate nitrogen. An equal amount of peat in the form of No. 52 gave off 28.9 mgm. of C as  $\text{CO}_2$  and produced 14.5 mgm. of nitrate nitrogen. The same amount of peat in horizon No. 53 gave off 51.7 mgm. of C as  $\text{CO}_2$  and liberated 15.6 mgm. of nitrate nitrogen. An equal amount of peat in the form of No. 54, or the sedimentary layer, produced 14.4 mgm. of C as  $\text{CO}_2$  and liberated 11.3 mgm. of nitrate nitrogen.

In other words, the seemingly marked differences would tend to disappear when compared on a dry basis of the peat undergoing decomposition. If a comparison is further made on the basis of ash-free peat, the results for the sedimentary peat (No. 54) would be further increased due to the high ash content of this horizon.

In general, one must conclude from these data that the rapidity of decomposition of the organic matter in the various peat layers is rather slow, as indicated by the slow evolution of carbon dioxide, when this is compared with the rapidity with which carbon dioxide is given off in the decomposition of fresh organic matter. However, in the decomposition of the peat there is rapid evolution of nitrogen in an available form. With the exception of the very lowest layers of older saw-grass peat, the ratio between the carbon liberated as carbon dioxide and the nitrogen formed as nitrate is very narrow, almost 1.3 to 1 in the case of sedimentary peat and 2-3.3 to 1 in the upper layers of saw-grass peat. In the case of ordinary soil organic matter the ratio of the carbon liberated as  $\text{CO}_2$  to the nitrogen formed as nitrate is about 10:1, or very similar to the ratio of carbon to nitrogen in the soil organic matter itself (8).

These results tend to indicate that the nitrogenous complexes, in the Everglade peat at least, undergo much more rapid decomposition than the non-nitrogenous organic complexes. In other words the very definite ratio between the carbon to nitrogen has not been established as yet when compared with the organic matter in normal field soils. What will happen in these peats upon prolonged cultivation still remains to be shown. It is quite possible that the available nitrogen may be rapidly liberated and the carbon-nitrogen ratio actually widened. This may bring about later the actual need for nitrogen fertilization in an injudicious management of the soil. The economic problem might then become somewhat similar to that of the high-moor peats, where there was only little nitrogen to begin with.

To be able to compare the decomposition processes that would take place in these peat formations when brought to a lower moisture content, and to

have a more correct basis for comparison of the rapidity of decomposition of the organic matter in the different horizons, the various peat samples were partly air dried, then adjusted to the same moisture content; thus, 75 grams of material taken for the decomposition studies contained 25 grams of dry matter and 50 grams of water, giving a moisture content of 66.7 per cent. These preparations were placed in aeration flasks and incubated as before. Both ammonia and nitrates were determined in the peats at the end of the incubation period. The results reported in table 4 are quite similar to those obtained with the naturally moist peat. The younger saw-grass peat and surface layers have decomposed more rapidly than the lower layers, especially the older fibrous peat. These results confirmed the previous observations concerning the very narrow ratio between the carbon liberated as  $\text{CO}_2$  and the nitrogen made available as ammonia and nitrate.

The rapid liberation of the nitrogen in the peat in an available form no doubt accounts for the high fertility of these bogs when they are drained and brought

TABLE 4

*Evolution of  $\text{CO}_2$  and formation of nitrate from different layers of an Everglade peat profile, using 25-gm. portions of dry material and 200 per cent moisture*

NUMBER OF LAYER	TOTAL $\text{CO}_2$ EVOLUTION (MGM. C)				NITROGEN LIBERATION IN 32 DAYS (MGM.)		
	5 days incubation	12 days incubation	26 days incubation	32 days incubation	$\text{NH}_3$	$\text{NO}_3\text{-N}$	Total N
50	11.5	23.9	44.6	54.8	4.82	13.20	18.02
52	16.9	28.6	44.4	51.8	2.45	23.24	25.69
54	3.8	7.0	13.6	15.7	1.25	5.70	6.95
56	2.9	6.7	16.2	21.1	1.20	3.45	4.65

under cultivation. Although certain differences are found both in the chemical composition and in the rapidity of decomposition between the typical saw-grass peat and the sedimentary or plastic peats, these differences are not sufficient to explain why the latter type of peat (as found around Lake Okeechobee) should prove to be much more fertile than the saw-grass peats themselves. It is suggested, as a result of these studies that the insufficient fertility of saw-grass peats as compared with sedimentary peats should be looked for in some other factor rather than in the nature and abundance of the nitrogenous complexes in the two types of peat or in the rapidity of their relative decomposition and liberation of the nitrogen in an available form.

Allison and his associates (1) have shown that the addition of copper to saw-grass peat greatly stimulates the growth of cultivated crops; however, copper has no effect upon the microbiological activities in the peat itself. This shows further that we are dealing here with another limiting factor in the growth of plants on these two types of peat.

Smith (9) actually succeeded in demonstrating the existence in certain



types of peat of a substance which is distinctly toxic to plant growth; this toxicity or injurious action can be overcome by the use of copper. It still remains to be shown of course whether this specific injurious substance exists only in the one type of peat and not in the other. At least one fact remains certain that if such a substance exists in the saw-grass peat and not in the sedimentary type peat, its action upon the growth of certain higher plants must be much more marked than upon the growth of certain microorganisms in the soil.

TABLE 5  
*Moisture content, reaction, ash, and nitrogen content of sedimentary peat profile*

NUMBER OF SAMPLE	DEPTH FROM SURFACE OF PEAT	MOISTURE CONTENT OF FRESH MATERIAL	REACTION OF FRESH MATERIAL	NITROGEN CONTENT OF DRY MATERIAL
	cm.	per cent	pH	per cent
80	1-30	97.82	7.50	3.40
81	30-60	97.75	7.55	3.00
82	60-120	90.90	7.25	2.41
83	120-180	91.46	7.06	2.48
84	180-240	95.45	7.15	2.56
85	240-300	94.23	7.28	2.67

TABLE 6  
*Chemical composition of the various layers of the sedimentary peat profile*  
(Percentage calculated from total of dry material)

NUMBER	ETHER SOLUBLE FRACTION	ALCOHOL SOLUBLE	HOT WATER SOLUBLE	HEMICEL- LULOSE	LIGNIN- LIKE COM- PLEXES	PROTEIN	ASH	SUM
80	0.12	0.15	1.09	13.96	27.02	21.25	18.81	82.40
81	0.16	0.37	0.94	12.87	32.50	18.75	24.44	90.03
82	0.41	0.61	0.71	4.17	35.19	15.06	39.56	95.71
83	0.59	1.02	0.60	2.40	52.58	15.50	23.30	95.99
84	0.84	0.96	0.59	4.01	63.22	16.00	8.79	94.41
85	0.62	1.55	0.76	3.34	51.55	16.69	14.73	89.24

The second peat profile from Florida, namely, the allochthonous or Gytjtja peat from Mud Lake was also subjected to a chemical analysis, using the methods previously (10) outlined. The results are given in tables 5 and 6.

The whole profile was practically free from cellulose, with the exception of mere traces in the upper layers. The low content of ether, alcohol, and water soluble materials, the high content of ash, protein and lignin-like complexes characterize this pure sedimentary profile as well as they do the sedimentary layers of other profiles. It is interesting to compare these figures with the composition of the Gytjtja layers ( $A_6$ ) of the Newton peat profile (11), which had 0.36 per cent ether-soluble material, 1.24 per cent water-soluble material, no cellulose, 5.92 per cent hemicellulose and 9.81 per cent protein. The

difference in the ash content in the different sedimentary peats will explain, to a large extent at least, certain of the differences in composition.

This formation was found to represent a number of highly interesting chemical and microbiological phases and it is our hope to come back to it later, for more extensive studies.

#### SUMMARY

1. The results of chemical analyses of an Everglade peat profile and a pure sedimentary (Gyttja) lake profile are reported.

2. The Everglade peat profile contains small amounts of cellulose only in the upper layers of saw-grass peat but none in the plastic and lower fibrous layers.

3. The chemical composition of the saw-grass layers of the Everglade peat is similar to other lowmoor peats. It is characterized by a low content of ether-, alcohol-, and water-soluble constituents, a medium hemicellulose and ash content and a considerable protein and lignin content. The plastic or sedimentary layer, however, is characterized by a low hemicellulose content and an exceptionally high ash content. The content of lignin-like complexes and proteins, when calculated on an ash free basis, are similar to the saw-grass peat layers.

4. In the decomposition of both the saw-grass and sedimentary layers of the Everglade peat, there is a rapid evolution of nitrogen in an available form and its accumulation as nitrate. The ratio between the carbon liberated as carbon dioxide and the nitrogen changed to nitrate is very narrow (1.3:1 to 3.3:1), much more so than in the decomposition of organic matter in ordinary soil.

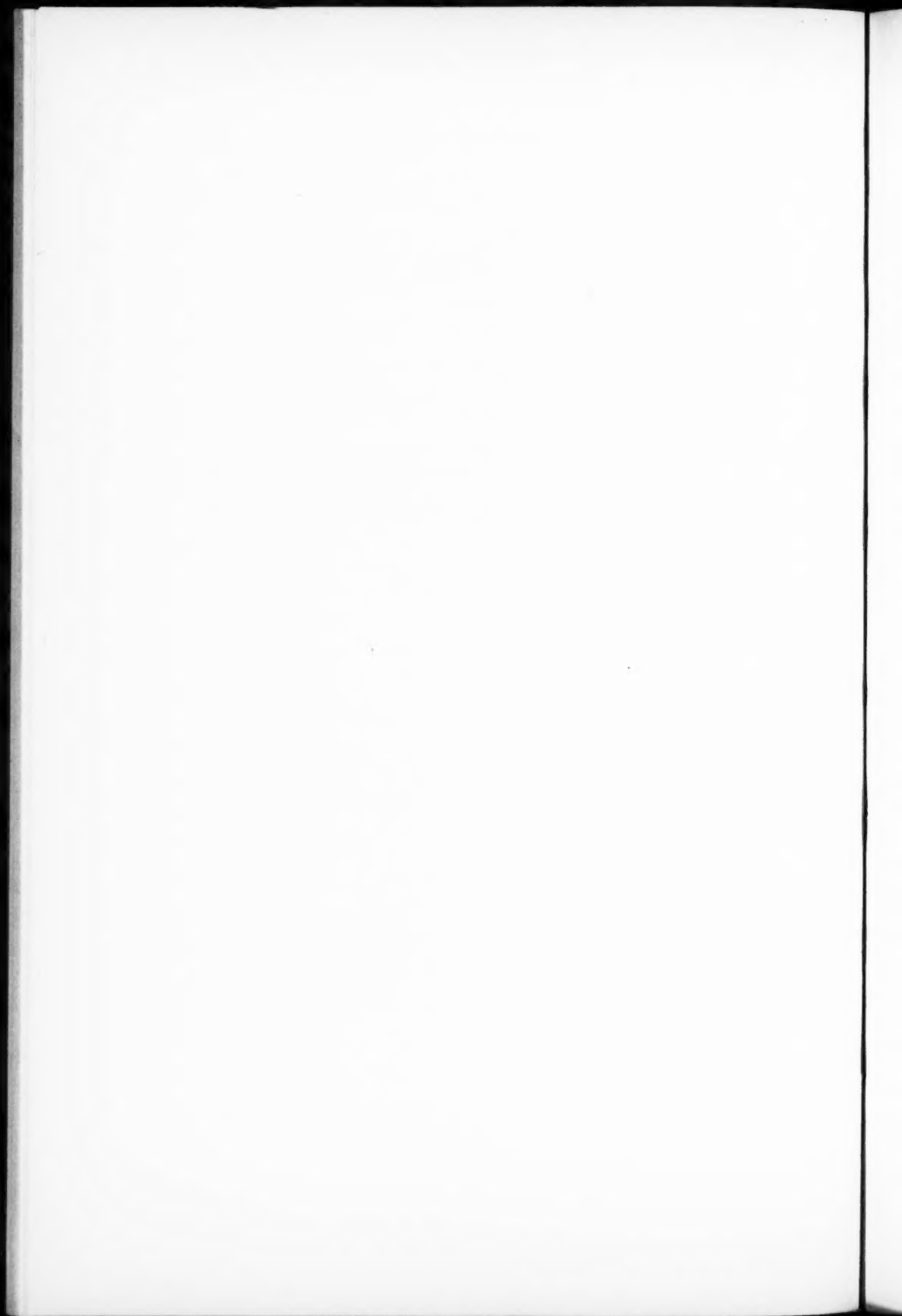
5. There is no marked difference in the rapidity of liberation of nitrogen in an available form in the saw-grass and sedimentary layers of the Everglade peat profile. Any difference in fertility of soils produced by drainage and cultivation of the saw-grass and sedimentary types of peat and any favorable effect of copper upon plant growth should be looked for not in any difference in the activities of microorganisms or liberation of nitrogen from the organic complexes of the peat, but as due to some other factor.

6. An analysis of the chemical composition of a sedimentary peat profile taken from a lake in Florida, outside of the Everglade region, shows a similarity of this peat to sedimentary (Gyttja) layers of other peats, namely freedom from cellulose, a low content of ether-, alcohol-, and water-soluble constituents, a fairly high hemicellulose content, a high ash, protein, and lignin content.

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## MICROBIOLOGICAL ACTIVITIES IN THE SOIL OF AN UPLAND BOG IN EASTERN NORTH CAROLINA<sup>1</sup>

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In the autumn of 1924, Dr. B. W. Wells and the writer began an ecological study (22) of an upland grass-sedge bog located near Burgaw, Pender County, North Carolina. The vegetation on this area was decidedly unusual as compared with the type of vegetation found on most of the other parts of the coastal plain region of North Carolina, and pointed to an interesting complex of causal factors.

In connection with this ecological study the writer began the study of the microbiological activities of the soil of this upland bog. These soil studies were continued until the autumn of 1927 and form the basis of this paper.

### DESCRIPTION OF THE BOG AREA

*Location.* The bog, known locally as the "Big Savannah," is located about 2 miles north of Burgaw, Pender County, North Carolina. It is on a part of the Wicomico terrace of the coastal plain region. This terrace was raised from the ocean during the Pleistocene Age. The bog is located a few miles west of the eastern edge of this terrace, and is now 24 miles from the ocean.

*Size and topography.* The area covered by the main bog is about 2½ miles long by 1 mile in width, comprising about 1,600 acres. The general shape of the area is in the form of a capital Y.

The surface of the bog is 17.7 meters above sea level. Although the main part of the bog is almost entirely level, the surface is roughened by innumerable small tussocks and ridges which are rarely over 10 cm. high. Scattered over the area there are numerous ant-hills which are 15 to 20 cm. high.

The bog is one of the highest areas in Pender County. On every side of the bog we find the beginnings of small streams which have their origin in shallow swamps, only a few centimeters to a meter or two below the surface of the main part of the bog.

*Vegetation.* The bog has been called a savannah by local people, and more commonly the "Big Savannah." It is not, however, a true savannah, as a savannah is a grassland with trees on it, whereas the bog in question is

<sup>1</sup> Part of a thesis submitted to the faculty of Rutgers University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

almost free from trees. Its general aspect is rather that of a prairie. The plants on the bog are representatives of about one hundred species, mostly perennials. The dominant plants are grasses and sedges. Species of *Carex* are however entirely absent, and leguminous plants never occur on the bog proper. A complete list of the species of plants found on this area is given by Wells and Shunk (22).

The grass-sedge complex is kept on the bog due to frequent fires which occur almost once a year and keep the bog practically treeless.

*Soil.* The soil of the bog is of the type known as "Portsmouth very fine sandy loam." It has been formed from the very fine sand laid down in deep water 15 to 20 miles off shore, before the Wicomico terrace was raised above the level of the ocean. A partial mechanical analysis of the surface soil made by Wells and Shunk (22) gave the following:

	MILLIMETERS	PER CENT
Fine gravel.....	2 -1	0
Coarse sand.....	1 -0.5	0
Medium sand.....	0.5 -0.25	5.4
Fine sand.....	0.25-0.1	24.5
Very fine sand, silt, clay, etc.....	0.1 and less	70.1

The subsoil is a mottled gray and yellow very fine sandy clay to a depth of a meter and more.

The carbon content of the surface soil as found by analysis is 3.26 per cent of the dry weight of the soil. The total nitrogen content is 0.24 per cent, thus giving a carbon-nitrogen ratio of 13.6 : 1 as compared to the usual average 10 : 1 ratio of cultivated fertile soils.

One of the most marked characters of the soil of this upland bog is the thick dark humus layer. This layer is from 35 to 40 cm. thick in the middle of the bog. The greater part of the roots of the grasses and other plants is found in this dark soil layer, and it is doubtless due to the partial decomposition of these roots that this thick layer containing humus has been accumulated. In most other types of areas of the coastal plain region the humus layer is rarely over 10 cm. thick.

On account of the very level surface of the whole bog, and the practically impervious subsoil, the area has very poor drainage. Much of the rain that falls on the bog remains there until lost by evaporation, or by the transpiration of plants. For this reason anerobic conditions prevail in this soil for a considerable part of the time, each heavy rain nearly or completely filling the bog with water. Even in dry weather, on account of the fineness of the soil particles causing good capillary action, the upper part of the bog remains moist, except in such very unusual seasons as the summer of 1926, when the water table fell to a meter and more below the surface. During this time the surface became quite dry.



## HISTORICAL

So far as the writer has been able to learn, there has been little work done previously on the microbiology of the soils of upland bogs such as the soil of the "Big Savannah," and other grass-sedge bogs of eastern North Carolina. There have been made in North Carolina, however, some studies giving an idea of the extent of ammonification of nitrification in soils similar to the soil with which this paper deals.

Stevens and Withers (13) in 1909 in a series of tests of ammonification and nitrification, collected among other samples, one from a "savannah" in Craven County, N. C. This sample was tested for its ability to cause nitrification when inoculated into a suitable sterilized soil to which ammonium sulfate had been added. A further test was made to determine its ability to nitrify ammonium sulfate added to the soil itself, and incubated at optimum moisture concentration for four weeks. The results of the tests were as follows: (a) No nitrification occurred in the soil containing ammonium sulfate after inoculation with an infusion from the "savannah" soil. (b) When ammonium sulfate was added to the soil itself, no nitrate was found after four weeks of incubation.

Willis (24) has studied the relations existing between nitrification and acidity in certain muck lands of eastern North Carolina. In cut-over swamp land that has been drained, Willis found nitrate nitrogen in soil with an acidity as great as pH 3.62. He found that a sort of equilibrium existed about pH 4.0. If a small amount of lime was added, and the reaction made less acid than pH 4.0, nitrification was accelerated and the production of nitric acid from the large supply of organic nitrogen on hand again brought the pH to approximately 4.0.

## EXPERIMENTAL WORK

The experimental work was carried out by studies of the following:

- I. The numbers of bacteria, actinomycetes and fungi in the soil under different conditions of treatment.
- II. Effect of different soil treatments on the formation of ammonia and nitrates.
- III. Rate of activities of soil microorganisms under different treatments as evidenced by the amount of carbon dioxide evolved.
- IV. Character and amount of the soil organic matter.
- V. Decomposition of cellulose added to the soil.
- VI. Nitrogen fixation.

*I. Numbers of microorganisms*

Eight samples of soil were taken to a depth of about 15 cm., sifted through a 2 to 3 mm. sieve and composited. The moisture content of this soil when taken to the laboratory was 9.9 per cent on the basis of dry soil. By the use of Hilgard cups it was found that the water holding capacity of this soil was 63.1 per cent on the basis of dry soil.

The moisture content of the soil was brought to 60 per cent of its water holding capacity by the addition of distilled water. Twelve pots were then filled by using 2500 gm. of moist soil per pot. Each of the pots was then inoculated with a suspension of soil from a local greenhouse (State College, Raleigh, N. C.) mixed with soil from a cultivated field near the "savannah bog."

The soil in the pots was treated as follows:

*Pot number*

- |    |  |
|----|--|
| 1  | Untreated  |
| 2  | Untreated  |
| 3  | Plus 0.5 per cent of calcium carbonate                                     |
| 4  | Plus 0.5 per cent of calcium carbonate                                     |
| 5  | Plus 0.10 per cent of sodium nitrate                                       |
| 6  | Plus 0.10 per cent of sodium nitrate                                       |
| 7  | Plus 0.5 per cent of calcium carbonate and 0.10 per cent of sodium nitrate |
| 8  | Plus 0.5 per cent of calcium carbonate and 0.10 per cent of sodium nitrate |
| 9  | Sub-optimum moisture, 40 per cent of saturation                            |
| 10 | Sub-optimum moisture, 40 per cent of saturation                            |
| 11 | Flooded  |
| 12 | Flooded  |

These pots were kept in the incubator room at a temperature of approximately 25°C. for a little over four months, and then kept at room temperatures for the remainder of the experiment.

Throughout the course of the experiment samples of soil from the different pots were plated at eight different intervals and the numbers of bacteria, actinomycetes and fungi were determined. The media used were acid agar [Waksman (15)] for the determination of the fungi, and albumen agar [Waksman and Fred (16)] for the determination of the bacteria and actinomycetes.

Table 1 gives a summary of the averages of the numbers of microorganisms found in the eight different platings, and also the pH of the soils at the close of the experiment. The reaction of the soils was determined by the use of the quinhydrone electrode. Table 2 is given to show the fluctuation in numbers of the bacteria in the case of soil with optimum moisture, with lime added and optimum moisture, and under flooded conditions.

The addition of calcium carbonate caused a great increase in the numbers of bacteria. As shown by figure 1 in which the numbers of the bacteria in the soil that had been limed and in the untreated soil are compared, at the end of the first 26 days the bacterial numbers are about one hundred times as high for the limed soil as for the unlimed. From that time until the 181<sup>st</sup> day, there is a fairly large uniform decrease in the numbers in the limed, as compared with a slight decrease for the unlimed soil. From the 181<sup>st</sup> day to the 291<sup>st</sup> day, the numbers show a steady rise for the limed soil, whereas the numbers in the untreated soil show merely a slight increase.

It is evident that many of the organisms present in the soil at the beginning, or added in the inoculum, found conditions favorable in the limed soil, and

thus gave the tremendous initial increase in the numbers. However, after a few weeks they decreased quite rapidly, due perhaps to the using up of certain of the more readily available food materials. This decrease seems

TABLE 1

*Summary of averages of numbers of microorganisms present per gram of dry soil in the pots during the period October 29, 1926 to August 16, 1927*

TREATMENT	POT	BACTERIA AND ACTINOMYCES	ACTINO-MYCES	FUNGI	pH AT CLOSE OF EXPERIMENT
None.....	1	2,558,000	327,000	81,000	5.2
	2	2,565,000	388,000	87,000	5.25
Plus lime.....	3	109,200,000	474,000	74,000	7.4
	4	126,900,000	512,000	77,000	7.3
Plus nitrate.....	5	7,860,000	513,000	138,000	4.7
	6	2,208,000	431,000	107,000	4.95
Plus lime and nitrate.....	7	104,900,000	392,000	92,000	7.3
	8	118,070,000	449,000	94,000	7.4
Sub-optimum moisture.....	9	1,588,000	389,000	80,000	4.8
	10	1,482,000	284,000	93,000	4.85
Flooded.....	11	1,243,000	410,000	57,000	5.6
	12	1,029,000	425,000	55,000	5.75

TABLE 2

*Fluctuation in numbers of bacteria per 1 gram of dry soil in drained, drained and limed, and in flooded soil*

INCUBATION	DRAINED (AVERAGE OF POTS 1 AND 2)	DRAINED AND LIMED (AVERAGE OF POTS 3 AND 4)	FLOODED (AVERAGE OF POTS 11 AND 12)
<i>days</i>			
26	1,935,000	233,250,000	1,050,000
61	1,963,000	193,624,000	533,000
88	1,450,000	143,650,000	680,000
116	1,545,000	136,725,000	415,000
150	760,000	49,650,000	652,000
181	2,796,000	22,600,000	1,012,000
239	2,825,000	60,330,000	910,000
291	4,320,000	101,833,000	995,000
Averages for period....	2,199,000	117,708,000	781,000

not to have been due to a decrease in the amount of mobilized nitrogen, for the amount of ammoniacal and nitrate nitrogen was actually greater during the period of bacterial depression than earlier in the experiment when the numbers of bacteria were higher.

The lowest part of the curve in figure 1 for the limed soil is correlated with the temperature conditions under which the soil was kept. On March 12, 1927, on the 134<sup>th</sup> day of the experiment, the pots were removed from the incubator room where they had been kept at a temperature of approximately 25°C. The soils were then transferred to glass jars and transported to New

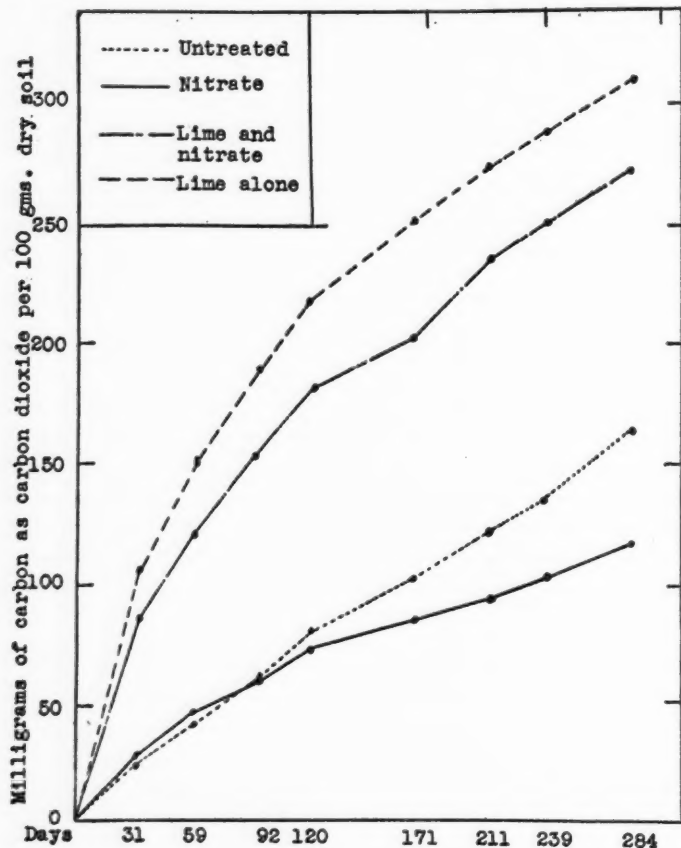


FIG. 1. NUMBERS OF BACTERIA IN UNLIMED AND LIMED SOILS BASED ON THE AVERAGES OF POTS 1 AND 2, AND OF POTS 3 AND 4

Jersey in an open car. On their arrival at the New Jersey Experiment Station they were transferred to pots and kept in the laboratory at room temperatures for the remainder of the experiment. During the trip from Raleigh, N. C. to New Brunswick, N. J. the temperature dropped to around the freezing point, or below, and the room temperature at New Brunswick was nearer

20° than 25°C. until well into the summer. Later in June, July, and August the general increase in the summer temperature brought the room temperature to a higher figure, the rise in temperature at this time being correlated with the rise in bacterial numbers.

In a second pot experiment soil was treated with 0.10 per cent of ammonium sulfate and 1 per cent of calcium carbonate and kept in a pot for approximately 18 months. It was kept at optimum moisture concentration for three months and then allowed to become quite dry (about 20 per cent of saturation).

Table 3 gives the numbers of microorganisms present as shown by plating this soil at the beginning and end of the 18-month period.

After 18 months of incubation, whitish spots were noticed on the rather dry lumps of soil. If a little of this whitish material was scraped from a lump of soil, moistened with a drop of water, and examined microscopically, either unstained or stained by the addition of a drop of methylene blue, it was found that there was an abundance of actinomycetes growth. Some of the whitish material was scraped off, fixed to the slide with a dilute agar solution, and then stained with erythrosin. Large masses of actinomycetes were seen. The masses became stained in such a way as to look very similar to large groups of spherical or short rod-shaped bacteria. In fact, if one had not seen the unstained preparations, he might readily have considered these masses of actinomycetes as bacteria, and counted them as such.

As shown by table 3, the plate method using albumen agar gave only 300,000 actinomycetes per gram in this soil, although microscopic examination showed them to be very numerous. Two suggestive conclusions may be drawn from the examination of this sample of soil: (a) When stained preparations of soils are examined, the masses of so-called short rod-shaped or spherical bacteria may be in reality masses of actinomycetes; and (b) large numbers of actinomycetes sometimes fail to grow on the albumen agar used for making plate counts.

According to the results of the microscopic examination, although it was not found possible to determine even approximate numbers, the number of actinomycetes must have increased greatly in this soil under its treatment. The soil sample at the end of the period had a pH of 7.53, determined by the quinhydrone method. The slightly alkaline reaction together with the dry condition of the soil with its accompanying improved aeration, was thus shown to favor the abundant development of actinomycetes.

## II. Ammonia and nitrates

Samples were taken at intervals from the series of pots in which the fluctuation of the numbers of microorganisms was studied, and determinations were made of the ammoniacal and of the nitrate nitrogen present. The amount of ammonia was determined by leaching the soil with half-normal potassium chloride solution, adding magnesium oxide and distilling the ammonia into standard acid. Nitrates were determined by the usual phenoldisulphonic acid method.

The fluctuations in the amounts of ammoniacal and nitrate nitrogen from time to time are given in table 4. It is interesting to note that no nitrification occurred in the soil of any of the pots except those to which lime had been

TABLE 3  
*Microorganisms in soil receiving ammonium sulphate and lime*

Figures based on 1 gram of dry soil

INCUBATION PERIOD	BACTERIA AND ACTINOMYCES	ACTINOMYCES	FUNGI
None.....	1,103,000	20,000	13,400
18 months.....	25,900,000	300,000	40,000

TABLE 4  
*Milligrams of N as ammonia and as nitrate per 100 grams dry soil*

INCUBATION	UNTREATED (AVERAGE OF POTS 1 AND 2)		LIMED (AVERAGE OF POTS 3 AND 4)		NITRATE ADDED (POTS 5 AND 6)		LIMED AND NITRATED ADDED (POTS 7 AND 8)		SUB-OPTIMUM MOISTURE (POTS 9 AND 10)		FLOODED (POTS 11 AND 12)	
	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>
days												
62	0.8	None	8.2	None	5.5	15.7	7.2	15.2	0.7	None	0.8	None
118	1.8	None	3.2	5.8	7.3	20.2	10.3	21.1	1.1	None	1.5	None
181	2.3	None	0.3	12.1	8.8	14.9	1.3	23.7	1.5	None	2.7	None
237	2.9	None	0.4	11.2	9.5	12.1	0.2	22.2	1.6	None	2.9	None
293	4.7	None	0.7	13.3	11.2	11.7	0.6	24.7	2.2	None	4.3	None

TABLE 5  
*Milligrams of carbon evolved as carbon dioxide per 100 grams of dry soil in a period of 284 days*

FLASK NUMBER	TREATMENT	C AS CO <sub>2</sub> EVOLVED
1	Untreated	164.0
2	Untreated	162.3
3	Limed	225.4
4	Limed	234.7
5	Sodium nitrate added	114.6
6	Sodium nitrate added	120.8
7	Limed and nitrate added	194.8
8	Limed and nitrate added	201.4
9	Sub-optimum moisture	111.8
10	Sub-optimum moisture	121.9
11	Flooded	107.8
12	Flooded	110.9

added. In pots 3 and 4, which had been limed, but had been given no addition of nitrates, the soil showed an accumulation of 14 mgm. of nitrogen as nitrate per 100 gm. of dry soil. Since 22.7 mgm. of nitrogen were added to pots 7 and 8 per 100 gm. of soil, one would have thus expected to find some-



thing like 36.7 mgm. of nitrate nitrogen per 100 gm. of soil, unless some of it had been reduced or lost. On the other hand, the average amount of mobilized nitrogen in the soil of pots 7 and 8 at the close of the experiment was 25.3 mgm. per 100 gm. of soil. Undoubtedly, a considerable amount of

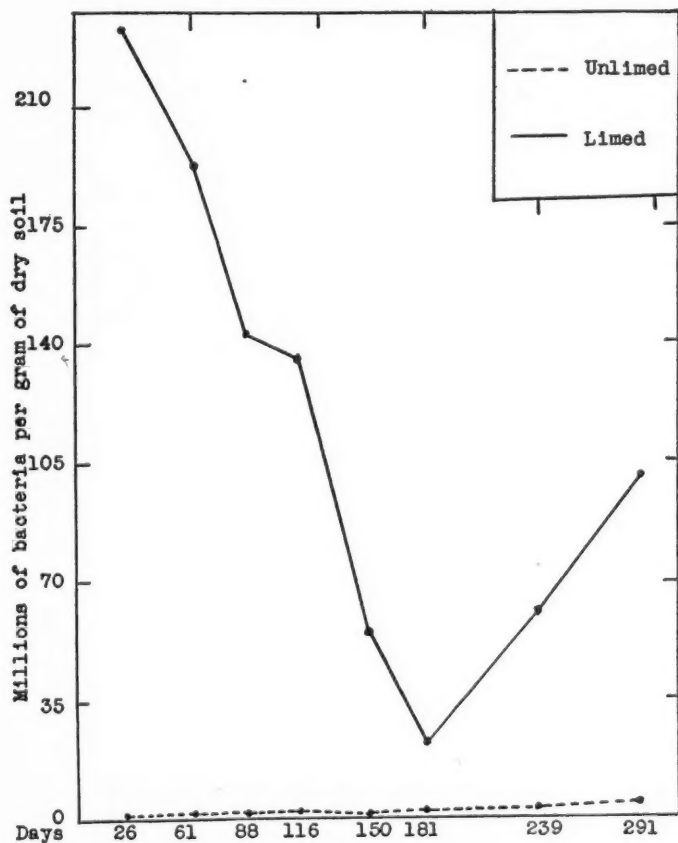


FIG. 2. EFFECT OF LIMING AND ADDITION OF NITRATES ON THE EVOLUTION OF CARBON DIOXIDE

the ammonia, nitrate nitrogen, or of both must have been immobilized by being built up into microbial protoplasm.

### III. Carbon dioxide evolution

From each of the 12 pots used for determination of numbers of microörganisms, and for the study of ammonia and nitrate formation, enough moist

soil to be equivalent to 100 gm. of dry soil was taken and placed in a flask. These flasks were connected with aeration apparatus and the evolution of carbon dioxide determined from week to week by the method of Waksman and Starkey (19).

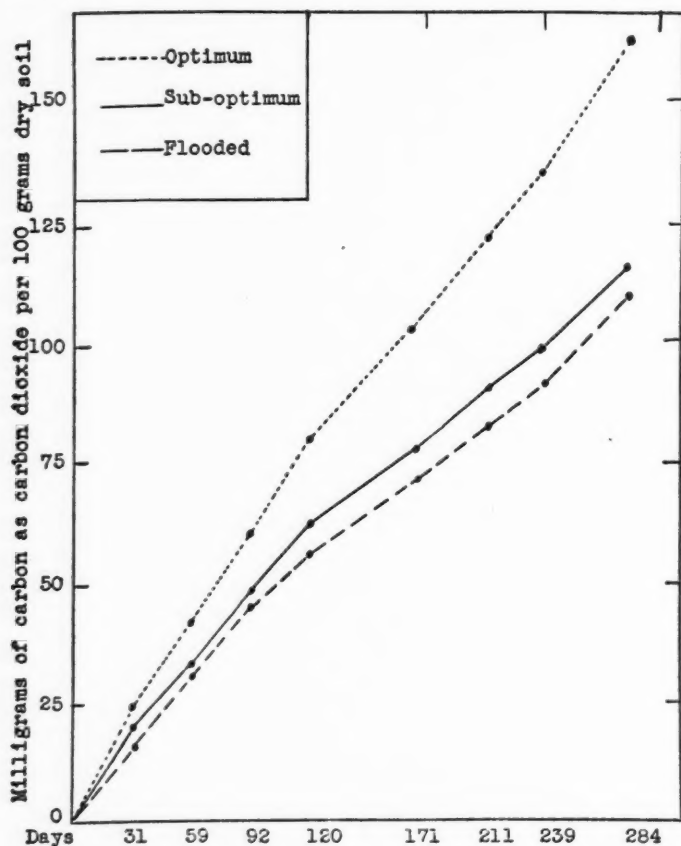


FIG. 3. EFFECT OF AMOUNT OF MOISTURE ON EVOLUTION OF CARBON DIOXIDE

Table 5 gives the total carbon evolved as carbon dioxide per 100 gm. of soil in each of these flasks in a total period of 284 days. In those soils to which lime had been added, the amount of residual  $\text{CO}_2$  in the lime was determined and the amount liberated due to interaction with soil acids was deducted from the totals. The figures for carbon dioxide evolved represent only that part which is attributable to the activity of microorganisms.

Figure 2 shows the effect of liming and addition of nitrate on the evolution of carbon dioxide, and figure 3 the effect of amount of moisture in the soil on the amount of  $\text{CO}_2$  evolved.

Liming the bog soil caused a considerable increase in the loss of carbon as carbon dioxide. This increase of evolved carbon dioxide was correlated with a large increase in the numbers of bacteria and also of actinomycetes present.

The addition of sodium nitrate to furnish available nitrogen for microbial use was shown to have a depressing effect on the rapidity of carbon dioxide production. As shown by figure 2, for nearly three months the amount of carbon dioxide was slightly in excess of that evolved from the untreated soil, but after about eighty days the amount of carbon dioxide dropped considerably below the amount from the untreated sample. When lime was added in addition to the nitrate, there was also a depressing effect so far as evolution of carbon dioxide was concerned.

The depressing effect on carbon dioxide evolution is probably due to a slight toxicity resulting from the amount of nitrate used.

#### *IV. Soil organic matter studies*

Following the methods worked out by Waksman (20), studies were made of the soil organic matter of the soil of the upland grass-sedge bog. The soil samples were extracted with hot alkali, both after and before treatment with hydrochloric acid. Extractions were made as follows: 50 gm. of dry soil were treated with 100 cc. of 1 per cent HCl solution and the acid allowed to act over night. The supernatant liquid was then decanted on a filter, and the soil washed with an additional 100 cc. of the HCl solution and then with about 20 cc. of water, and transferred to the filter. The soil was removed from the filter and dried in the oven. To each of the samples of dry soil, either with or without the previous acid treatment, were added 100 cc. of 2 per cent NaOH solution, and extraction carried out in the autoclave at 15 pounds pressure for 30 minutes. The supernatant liquid was decanted on a filter, and the residue, after washing with water, was then added to the filter. A second extraction was made by using 100 cc. of 4 per cent NaOH in the autoclave for 30 minutes. The extract was then decanted on the same filter and the soil washed with 50 cc. of water and transferred to the filter. The filter was washed with hot 1 per cent NaOH, and then with hot water. The filtrate was treated with an excess of 1-1 HCl and boiled for 20 minutes after which the precipitate, the so-called  $\alpha$ -fraction, was filtered on a weighed paper, dried, and weighed. The filtrate after the removal of the  $\alpha$ -fraction was treated with sufficient NaOH solution to bring the reaction to approximately the neutral point, and the precipitate, the  $\beta$ -fraction, filtered off, dried and weighed.

The total nitrogen in the  $\alpha$ - and  $\beta$ -fractions was determined by the Kjeldahl method, and the total carbon by the method of Waksman and Starkey (18).

Table 6 gives the amount of the various fractions and their nitrogen and carbon content.

The preliminary treatment with 1 per cent HCl solution for a period of nearly twenty-four hours had little effect on the amount of  $\alpha$ -fraction extracted by the alkali. This was in accord with the findings of Waksman (20) who reports even a slightly diminished yield of  $\alpha$ -fraction following treatment with hydrochloric acid. The acid treatment did, however, increase the amount of the  $\beta$ -fraction that was extracted by the alkali.

Of the total organic matter in the soil the  $\alpha$ -fraction yielded almost 20 per cent. The percentage of nitrogen in the  $\alpha$ -fraction was found to be close to 3 per cent. This percentage of nitrogen in the  $\alpha$ -fraction agrees closely with that found by others, Waksman (20) and Joseph and Whitfeild (11).

The carbon content of the  $\alpha$ -fraction was found to be almost exactly 60 per cent. In comparison with this percentage of carbon, Joseph and Whit-

TABLE 6  
*Soil organic matter extracted by alkali solution from 50 grams of dry bog soil under 15 pounds pressure, both with and without previous treatment with hydrochloric acid*

SAMPLE	HCl TREATMENT	$\alpha$ FRACTION			$\beta$ FRACTION		
		Yield	Nitrogen	Carbon	Yield	Nitrogen	Carbon
		grams	per cent	per cent	grams	per cent	per cent
1	Plus	0.790	3.1	....	0.964	0.1	....
2	Plus	0.820	3.0	....	0.829	0.1	....
3	Plus	0.773	...	61.0	1.166	...	1.95
4	Plus	0.809	....	59.4	0.827	...	2.0
5	Minus	0.769	2.9	....	0.725	0.1	....
6	Minus	0.720	3.0	....	0.528	0.4	....
7	Minus	0.743	...	59.0	0.805	...	3.55
8	Minus	0.764	...	60.7	0.559	...	2.25

feild (11) found approximately 57 per cent carbon in the "humus" extracted from three different soils in Africa.

The ash content of the  $\alpha$ -fraction was found to be low, 1.4 to 2.2 per cent, and might have been lowered by repeated washing of the fraction with HCl. A low ash content was to be expected from the work of Waksman (20) and of Joseph and Whitfeild (11). These authors obtained an ash content of less than 1 per cent.

In ordinary fertile soils, the carbon-nitrogen ratio usually varies between 8:1 and 12:1 as determined by various analyses made by a number of investigators. This ratio is usually given as approximately 10:1.

The total carbon in the soil of the "savannah" bog was found to be 3.26 per cent, and the nitrogen 0.24 per cent, thus giving a carbon-nitrogen ratio of 13.6 : 1. This wide ratio is largely the result of the more or less continued anaerobic conditions in the soil of the bog. Under these conditions a large

part of the carbon-complexes are broken down to intermediate products instead of carbon dioxide, and the carbon is thus left in the soil. The lignins especially tend to accumulate, as they are attacked only very slowly by anaerobic microorganisms. Since the organic matter in the soil has this wide ratio of carbon to nitrogen, when more organic matter in the form of roots and leaves is added, the decomposition is slow because of the lack of nitrogen available for the use of the microorganisms. On account of the wide carbon-nitrogen ratio in the plant debris added to this soil, and the slow decomposition of this organic matter, the tendency is to widen rather than narrow the carbon-nitrogen ratio of this soil.

#### V. Decomposition of cellulose added to the bog soil

Two methods of approach were used in studying the decomposition of cellulose added to the soil in the form of filter paper: (a) by determining the

TABLE 7  
*Carbon as CO<sub>2</sub> evolved; microorganisms present, and mobilized N at close of experiment*

TREATMENT	MGM. OF C AS CO <sub>2</sub> EVOLVED PER 100 GRAMS DRY SOIL	BACTERIA PER GRAM DRY SOIL	FUNGI PER GRAM DRY SOIL	MGM. N AS NITRATE PER 100 GM. DRY SOIL	MGM. N AS AMMONIA PER 100 GM. DRY SOIL
Filter paper only.....	27.1	1,047,000	61,000	None	0.4
	28.9	749,000	76,000	None	0.4
Filter paper and 21.7 mgm. N as NO <sub>3</sub> added.....	55.3	579,000	1,500,000	17.2	1.7
	66.3	315,000	1,828,000	18.7	1.9

rate of carbon dioxide production, and (b) by determining the residual cellulose after a period of incubation.

1. *Carbon dioxide production by soil containing filter paper.* Samples of moist soil weighing 100 gm., with a moisture content of approximately 50 per cent of saturation, were placed in each of four flasks.

Nos. 1 and 2. One gram of filter paper added.

Nos. 3 and 4. One gram of filter paper and 100 mgm. of sodium nitrate added.

The flasks were filled and allowed to stand in the laboratory. At intervals during a period of 33 days determinations were made of the CO<sub>2</sub> evolved. At the close of the experiment determinations were made of the numbers of microorganisms present, and of the mobilized nitrogen. The results of these determinations are given in table 7.

The addition of nitrogen in the form of nitrate caused a speeding up of the decomposition of the cellulose as evidenced by the increase in evolution of carbon dioxide. This increase in rate of CO<sub>2</sub> production may be due to the increase in the fungous activity as indicated by the increase in the numbers

of fungi found, but may be due also to the activity of cellulose decomposing bacteria, which would not grow on the media used for making the microbial counts.

This experiment indicates that the rate of decomposition of cellulose in this soil is dependent on available nitrogen rather than on the types of organisms present.

2. *Residual cellulose after incubation.* Four pots were filled with 1,200 gm. of moist soil on September 18, 1926. The soil was from the same composite sample from which the pots in the pot experiment were filled. To each of the pots enough filter paper was added to be equivalent to 1.29 gm. of pure cellulose per 100 gm. of dry soil.

Pots 13 and 14. Soil contained a slight excess of water for 15 days; afterwards, optimum moisture.

Pots 15 and 16. Soil kept flooded throughout the course of the experiment.

TABLE 8  
*Residual cellulose in 100 grams of dry soil*

POT	TREATMENT	GRAMS OF CELLULOSE AFTER 204 DAYS	GRAMS OF CELLULOSE AFTER 310 DAYS
1	No cellulose added. Optimum moisture	0.0144	.....
2	No cellulose added. Optimum moisture	0.0192	.....
13	1.29 grams of cellulose added. Optimum moisture	.....	0.7610
14	1.29 grams of cellulose added. Optimum moisture	0.8248	0.7807
15	1.29 grams of cellulose added. Soil flooded	0.9780	0.8651
16	1.29 grams of cellulose added. Soil flooded	0.7920	0.6960

About seven months, 204 days, after the filter paper was added, enough moist soil was taken from each of the pots to be equivalent to 50 gm. of dry soil. Samples were taken also from pots 1 and 2 of the pot experiment for determining numbers of organisms etc., to be used as controls. Pots 1 and 2, containing the same soil as the pots with added cellulose, were kept at optimum moisture concentration and treated similarly to pots 13 and 14. The only essential difference was that pots 1 and 2 had received no filter paper.

The amount of cellulose present in these samples of soil was determined by the method given by Waksman and Heukelekian (17).

Three hundred and ten days after the experiment was begun, another set of determinations of residual cellulose were made on the soil from pots 13, 14, 15, and 16. Table 8 gives the amount of cellulose found in the different samples after 204 days and after 310 days. Slightly more of the cellulose was decomposed under aerobic than under anaerobic conditions.

Determinations of the ammoniacal and nitrate nitrogen present in the soils



in the pots showed very small amounts of ammoniacal nitrogen and no nitrates at all.

It is quite evident from the small amount of mobilized nitrogen in these samples, that it is the lack of available nitrogen, rather than the lack of cellulose decomposing organisms that accounts for the slow decomposition of cellulose in this soil. This conclusion is supported by the experiment on the rate of carbon dioxide production by soil with added cellulose both with and without the addition of sodium nitrate. In that experiment it was found that when nitrate was added, although the soil was not inoculated with any organisms, there was a marked increase in the rate of carbon dioxide production which indicated a speeding up of cellulose decomposition.

### VI. Nitrogen fixation

There are no legume plants growing on the "savannah" bog; hence, any fixation of atmospheric nitrogen must be due to non-symbiotic microorganisms. The fact that the upper layers of the soil contain as much as 0.24 per cent of nitrogen, and none is ever added by man, indicates that atmospheric nitrogen must furnish a large part of this. Since there are two groups of bacteria, aside from nodule bacteria, that normally fix nitrogen, the aerobic *Azotobacter* type, and the anaerobic *Amylobacter* type, search has been made for both types of organisms.

1. *Aerobic nitrogen fixing bacteria.* Silica gel plates were prepared in accordance with the method used by Waksman and Carey (21). After the gels had been thoroughly washed in running water and in boiled distilled water, they were dried very slightly in the oven at 60°C. As soon as the surface of the gel was nearly dry, each plate was impregnated with 1 cc. of the following nutrient solution:

Dibasic potassium phosphate.....	1 gm.
Magnesium sulfate.....	0.2
Sodium chloride.....	0.2
Calcium chloride.....	0.1
Ferric chloride (5 per cent).....	1 drop
Mannite.....	15 gm.
Distilled water.....	100

After the addition of the 1 cc. of the above nutrient solution to each of a number of plates of the gel, the plates were returned to the oven and kept there until the surface had become fairly dry. A little calcium carbonate was powdered over the surface, and the plates were then ready for inoculation.

The silica gels were inoculated with small bits of the upland bog soil and some of the plates with a pure culture of *Azotobacter*. The plates were then incubated in a moist chamber for almost four weeks.

*Azotobacter* colonies developed readily on the plates inoculated with the

pure culture, but failed to develop on any of the plates inoculated with the bog soil, thus indicating the absence of *Azotobacter*.

Several months later another batch of silica gel plates were inoculated with a fresh sample of the bog soil and gave the same negative results, even after 52 days of incubation. The reaction of the bog soil has been found to be close to pH 5.2, as determined by several tests with the quinhydrone electrode. According to the findings of others, Gainey (8, 9, 10) and Christensen (3, 4) *Azotobacter* would not be expected to be present in this soil on account of its reaction.

2. *Anaerobic nitrogen fixing bacteria.* Two series of experiments were carried out in regard to anaerobic nitrogen fixation, the first, to show the presence, or absence of organisms that could fix nitrogen under anaerobic conditions, and the second to try to find *B. amylobacter* and isolate it.

*Series 1.* A medium was prepared according to the following formula:

Distilled water.....	1000 cc.
Dextrose.....	20 gm.
Dibasic potassium phosphate.....	1
Magnesium sulphate.....	3
Sodium chloride.....	0.01
Manganous sulfate.....	0.01
Ferric chloride solution (10 per cent).....	3-4 drops
Calcium carbonate.....	10 gm.

To each of 6 tumblers 100 cc. of the above medium was added, and sterilized in the autoclave. Each of the solutions in the tumblers was inoculated with a 5 cc. aliquot of a 5-minute soil suspension (100 gm. of soil added to 200 cc. of sterile distilled water, shaken for five minutes, and allowed to settle slightly). Four of the tumblers were placed in a large dessicator jar, and the oxygen absorbed by the addition of pyrogallic acid and sodium hydroxide. Determinations of total nitrogen were made on the solutions and inoculum of two of the tumblers, and gave a nitrogen content of 0.3 mgm. per tumbler. After 28 days of incubation under anaerobic conditions, the solutions in the other four tumblers were analyzed for nitrogen content and gave the following results: 3.85, 4.58, 4.35, and 3.45 mgm., respectively.

Since the inoculated solution at the beginning of the experiment contained 0.3 mgm. of nitrogen, there was a fixation of an average of 3.75 mgm. of nitrogen per 100 cc. of the solution containing 2 gm. of dextrose sugar.

Another experiment, conducted in the same way as the above, showed a fixation of approximately 4.2 mgm. of nitrogen per 100 cc. of solution. These two experiments prove quite conclusively that organisms which can fix atmospheric nitrogen under anaerobic conditions, are present.

*Series 2.* The method used to attempt the isolation of *B. amylobacter* was essentially that of Burri (2). A number of tubes were filled about one-half full of a medium having the following composition.

Dibasic potassium phosphate.....	3 gm.
Magnesium sulfate.....	0.2
Sodium chloride.....	0.01
Ferrous sulfate.....	0.01
Manganous sulfate.....	0.01
Dextrose.....	10
Calcium chloride.....	0.01
Agar-agar, Bacto.....	15
Distilled water.....	1000

After this medium was melted and allowed to cool partially, dilutions of the bog soil were mixed with it by shaking, and the agar allowed to set. A plug of poisoned agar of the following composition was then added.

Distilled water.....	100 cc.
Mercuric chloride.....	0.02 gm.
Agar-agar, Bacto.....	1.0 gm.

After incubation several of the shake-agar tubes showed colonies which gave rise to gas formation. By running a hot wire down along the side of the agar in one of these tubes, and then by shaking, the cylinder of agar was removed almost intact, and placed in a sterile dish. By the use of a flamed knife, a colony which had just begun to show gas formation was cut out of the cylinder of agar and transferred to a tube of nitrogen-free nutrient solution. By connecting this tube to another tube filled with raw potato, anaerobic conditions were soon established in both tubes. After a few days of incubation, a microscopic examination made by the nigrosin method, revealed a number of typical *B. amylobacter* organisms. The culture was, however, not pure.

Silica gel plates were prepared in the same way as for the *Azotobacter* tests except that dextrose sugar was used instead of mannite. In two of the plates inoculated with bits of the "savannah" soil, *B. amylobacter* was found to develop colonies next to the bottom of the plate.

#### GENERAL DISCUSSION

In the study of the upland grass-sedge bog, one of the points of interest has been to discover, if possible, how the upland bog conditions are essentially different from those of a lowland bog. In an upland bog such as the one studied, although each rain may fill the soil more or less completely with water, yet standing water does not remain on the soil continuously. We have a period of several days during which the soil is practically, or entirely saturated, followed by a few days in which the upper layers of the soil become partially dry and there is more or less aeration. On the other hand in a lowland bog the soil would be saturated more continuously, at least for longer periods at one time.

The accumulation of so much organic matter in the soil, as compared with the amount of organic matter in cultivated soils in the vicinity of the bog is due to the interaction of several factors. In the first place it is dependent on the type of vegetation which is composed of grasses, sedges and various other herbaceous plants. These plants continue to dominate the area, because fire sweeps over the bog almost every year and effectively prevents the trees which start to develop, from becoming established and shading out the grasses, sedges, etc. The masses of roots and underground stems of the herbaceous vegetation are found chiefly in the upper 30 to 40 cm. of the soil. Then, due in part to the more or less continuous anaerobic conditions, with their influence on microbial activities, the dead roots and other underground parts are only partially decomposed, thus allowing an accumulation of so-called humus, or soil organic matter. The accumulation of this organic matter then increases the water holding capacity of the soil, and tends to keep the soil too wet for good aeration, which would permit the rapid activity of aerobic microorganisms, thus aiding in the further accumulation of the humus.

The entire absence of leguminous plants from the grass-sedge bog is probably correlated with the inability of the legumes to withstand saturated soil conditions for several days at a time. The absence of all legumes should not be attributed to the effects of acidity, as there are certain species of this group of plants that can and do grow in more acid soils, and legume nodule bacteria which can do well at higher concentrations of hydrogen-ions [Fred and Davenport (7)]. In the absence of leguminous plants, the source of the nitrogen in the soil must be largely the fixation of atmospheric nitrogen by non-symbiotic nitrogen fixing microorganisms. No nitrogenous fertilizer is ever added to this soil, as it is never cultivated, and yet the nitrogen content of the upper layers of the soil is rather high (0.24 per cent of the dry weight of the soil). The burning over of the bog causes a loss of most of the nitrogen that is in the aerial parts of the plants. This loss constitutes the principal loss of nitrogen, since there are no nitrates present to be lost by denitrification, or by drainage.

From the experimental work it has been found that *Azotobacter* species are not present. This absence of *Azotobacter* is no doubt due to the acidity of this soil and the anaerobic conditions. Just as found by Gainey (8, 9, 10), Christensen (3, 4), and others *Azotobacter* will not remain in a soil with a pH more acid than 6.0, whereas the pH of this soil is approximately 5.2. On the other hand, anaerobic nitrogen fixing bacteria can develop and fix nitrogen under more acid soil conditions, as found by Dorner (6). The anaerobic nitrogen fixing organism, *B. amylobacter*, has been found in several different samples of the soil by the use of silica gel plates and by other methods. The activities of this organism furnish undoubtedly the most important source of nitrogen in this bog soil.

Nitrate nitrogen has not been found in this soil in nature, although a number of samples have been tested. These samples were taken from different

places in the bog and at different times of the year over a period of about two years. One might suspect that the absence of nitrates and of nitrifying bacteria might be due to the acidity of the soil. However, the work of Willis (24) on muck soils of North Carolina, as well as of other workers elsewhere, Stephenson (12), Abbott, Conner and Smalley (1), White (23), and Temple (14), shows that nitrification does occur in acid soils. While the acidity of the soil is probably one of the factors hindering nitrification, it is not the only factor. When lime is added to the soil in pots in sufficient amounts to neutralize the acidity, and the soil is kept at optimum moisture concentration, nitrification will become evident after an interval of 6 to 10 weeks. It is advantageous to inoculate the soil thus drained and limed with a suspension of fertile soil, because it has been shown experimentally that nitrifying bacteria may be entirely absent from this soil.

The carbon-nitrogen ratio of the soil is quite wide, almost 14:1 as compared with the 10:1 to 12:1 ratio of fertile soils. This wide carbon-nitrogen ratio is undoubtedly one of the principal factors in the checking of nitrification. Just as found by Clark and Adams (5), a wide carbon-nitrogen ratio tends to check nitrification. Under these conditions the microorganisms use up most of the available nitrogen and store it in their microbial protoplasm; therefore, little is liberated as ammonia, and without ammonia we cannot have nitrification. When lime is added, and the soil is kept at optimum moisture concentrations, conditions are made favorable for the activity of aerobic bacteria and of actinomyces, and since the bacteria can get along with less nitrogen per unit of carbon consumed as energy than the fungi [Waksman and Heukelekian (17)], more nitrogen will be liberated as ammonia. When the soil reaction is neutral and there is ammonia present, nitrification will take place.

When cellulose in the form of filter paper was added to the soil, its decomposition was very slow, both under aerobic and anaerobic conditions. By using the rate of evolution of carbon dioxide as an index of the rate of decomposition of the cellulose, this slow decomposition was found to be due to the lack of available nitrogen, since when nitrate nitrogen was added there was a considerable increase in the rate of carbon dioxide production, accompanied by a large increase in the number of fungi present.

The cellulose added to the soil in nature by the plant residues, mostly roots and other underground parts, since the tops are usually burned every year, is largely decomposed. Cellulose determinations made on the soil from the bog showed a low percentage of cellulose.

If the soil is limed and allowed to become quite dry, actinomyces become active. Since these organisms are probably the principal microorganisms that can attack the  $\alpha$ -fraction of the soil organic matter, and they are hindered by lack of aeration and also by acidity, little decomposition of the  $\alpha$ -fraction can occur in the bog soil under the conditions found in nature. This leads to an accumulation of the lignins and nitrogen complexes comprising the  $\alpha$ -fraction.

The results of the experimental work indicate that if this upland bog could be properly drained and then limed, that the organic matter would be gradually decomposed with the liberation of nitrogen as ammonia. This ammonia would then be nitrified. However, since a considerable part of the nitrogen is tied up in the  $\alpha$ -fraction of the soil organic matter, that part of the nitrogen would become available very slowly, and it would probably take several years of cultivation before a satisfactory yield of any ordinary crop plant could be obtained.

#### SUMMARY

A study has been made of the microbiological activities of the soil of an upland grass-sedge bog, and the modifications of these activities produced by the addition of lime and of nitrogen salts. The effects of drainage and flooding have been studied also.

Nitrification does not occur in this soil under natural conditions. It may be made to occur by liming and drainage as determined by pot experiments, but only after an interval of 6 to 10 weeks.

No leguminous plants are found growing on the bog and yet the soil contains 0.24 per cent of total nitrogen. The source of this nitrogen may be to some extent atmospheric nitrogen fixed by non-symbiotic organisms.

*Azotobacter* species are not present on account of the soil acidity and anaerobic conditions, the pH of the soil being about 5.2.

*Bacillus amylobacter* has been found in several samples of the soil.

The addition of enough lime to the soil to neutralize the acidity results in a great increase in the numbers of bacteria, but does not appreciably change the numbers of fungi.

Liming the soil markedly increases the rate of carbon dioxide production by the soil microorganisms.

When the soil is saturated with water, or contains too little water, the rate of carbon dioxide production is reduced in comparison with soil at optimum moisture concentration.

The soil has a wide carbon-nitrogen ratio, 13.6 : 1, which tends to retard the decomposition of the soil organic matter.

When the soil is neutralized with lime and allowed to become quite dry, actinomycetes become very numerous. These give the soil a whitish appearance.

Cellulose added to the soil in the form of filter paper is very slowly decomposed on account of the lack of available nitrogen.

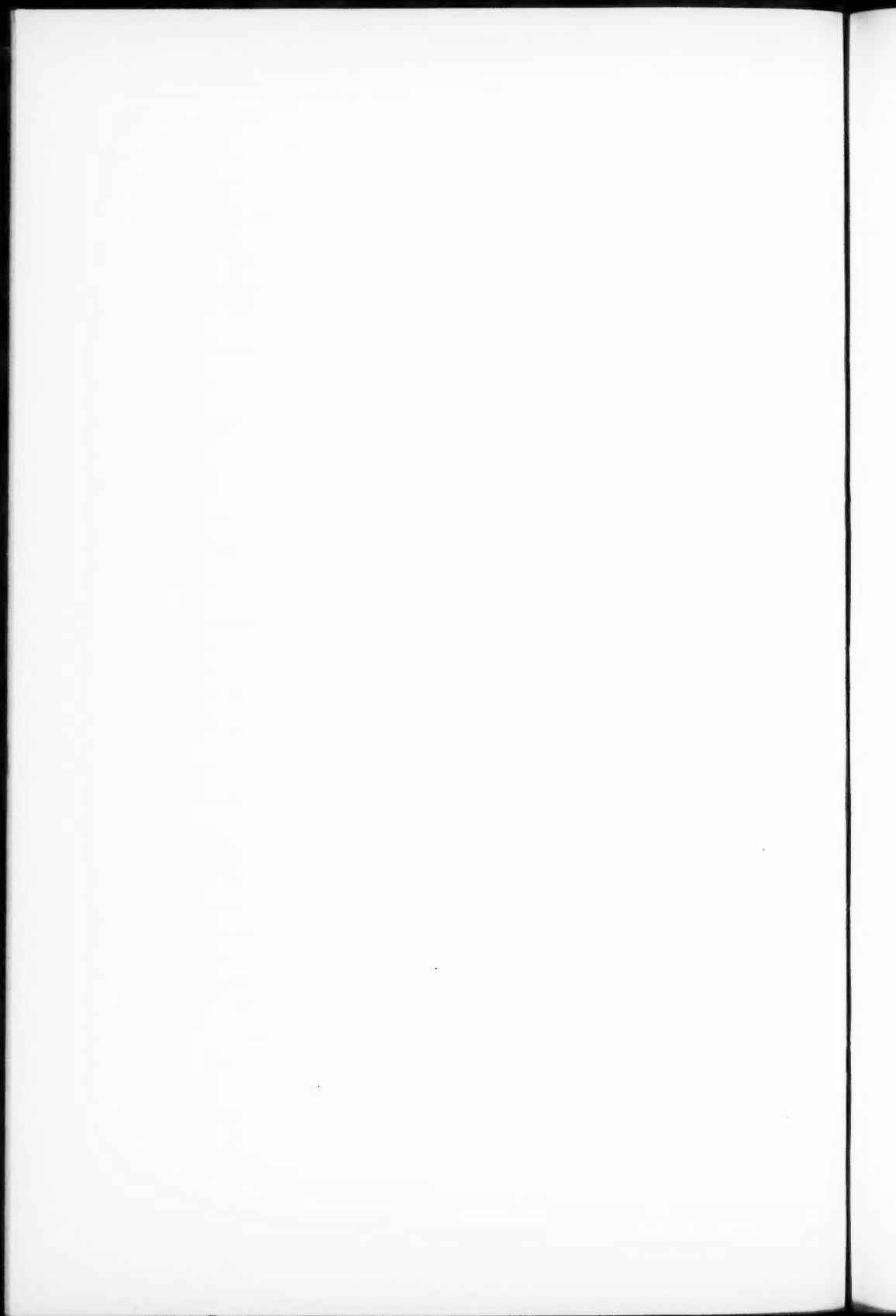
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# THE EFFECT OF MOISTURE CONTENT AND CROPPING ON EXCHANGEABLE CALCIUM AND MAGNESIUM, WITH PARTICULAR REFERENCE TO RICE SOIL<sup>1</sup>

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## INTRODUCTION

The subject of base exchange in soils has received considerable attention during the past five years. The nature of the exchangeable bases has been shown to have a very important bearing upon the physical properties of the soil. The effects of various factors which may influence the quantity and nature of the exchangeable bases have been investigated to a much less extent. Among these factors the influence of moisture content of the soil and of cropping upon the exchangeable bases has received little attention.

For several months the writer has been engaged in studies of soils utilized for the production of rice. The unusual moisture conditions under which rice is produced made it seem desirable to determine the effect of such conditions upon the exchangeable calcium and magnesium of the soil. The work reported in this paper was undertaken for the purpose of making more evident the effect of moisture content and cropping upon these exchangeable bases.

## REVIEW OF LITERATURE

The literature of the subject has been rather extensively reviewed in a number of recent publications and an exhaustive review is, therefore, unnecessary here. All writers are not agreed upon the nature of the base exchange complex and the manner in which the exchangeable bases are held in the soil. One group believes that these bases are held in chemical combination, perhaps largely as complex aluminosilicates, and that their replacement is accomplished by true chemical forces, subject to well defined chemical laws. In this group the works of Kelley and Brown (9), Burgess and McGeorge (2), and Kerr (10, 11) set forth some of the evidence supporting this view. Another group proposes that the exchangeable bases are adsorbed on the colloid particles of the soil. Hissink (6) advanced the supposition that the exchangeable cations

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<sup>2</sup> The thanks of the writer are hereby tendered to Mr. G. H. Banks, Assistant Director, In Charge of the Rice Branch Experiment Station at Stuttgart, Ark., who took the samples of Crowley silt loam and furnished the cropping history of this soil, and to Prof. C. K. McClelland for suggestions concerning the statistical treatment of the data.

are adsorbed as an external layer around the colloid particle, the inner layer being composed of the corresponding anions. This arrangement, in many respects, is similar to the Helmholtz double-layer, so familiar to students of colloid chemistry. Some recent publications by workers who apparently favor this view are those of Breazeale and Magistad (1) and Oden (16).

Some of the factors affecting the content of exchangeable bases of soils have been studied. Catherwood and DeTurk (3) have presented data showing the relation of maturity of soil type to exchangeable calcium and magnesium of various soil horizons. Humfeld (8) has shown the effect of fertilizer treatments upon the electrodialysable bases of the soil. Merkle (14) studied the effect of long-continued fertilizer treatments upon the exchangeable cations of the soil and found, in general, that fertilizers bearing the cations, Ca, Na, and K made significant increases in the amounts of these bases in exchangeable form. Ammonium sulfate treatment caused an increase in exchangeable hydrogen.

Martin (12) studied the effect of cropping (under conditions precluding leaching) upon the exchangeable bases of 13 California soils. He found continuous cropping for a period of twelve years, or cropping for two years with an intervening fallow period of 10 years, had no appreciable effect upon total exchangeable bases. Calcium and magnesium, comprising 90 per cent of the total exchangeable bases, were not changed in quantity. While it did not appreciably change the figures for total bases, the exchangeable potassium was decidedly reduced by two to twelve years of cropping with barley.

Ogg and Dow (17) found decidedly more exchangeable calcium in cultivated soils, as a group, than in "woodland," "hill or heath" soils, or soils "long unploughed."

#### DESCRIPTION OF SOILS

The rice soil investigated is classified as Crowley silt loam. It is the typical prairie soil of the rice section of Arkansas and is characterized by its very flat topography. The surface soil is a gray silt loam, of which silt comprises nearly 75 per cent and silt and clay together constitute 90 per cent. Underlying this at a depth of 20 to 24 inches is a very tight, highly plastic layer almost impervious to water, a very important feature of a soil well adapted to rice culture. Two horizons of this soil were studied, the first comprising the surface 7 inches and the second the 7 to 14 inches sub-surface layer. The reaction of the surface layer samples varied from pH 7.0 to 7.3, while the sub-surface layer samples showed reactions varying from pH 6.5 to 6.9.

The soil used for the study of effect of moisture content and that used in the study of effect of cropping is classified as Clarksville silt loam. It contains four-fifths as much silt and clay as the Crowley silt loam, possesses a more open structure, and has no impervious layer in the subsoil. It is derived largely from non-magnesian limestone, and has been subjected to fairly heavy cropping

and rather extensive leaching for a long period of time. The reaction of the samples from this soil varied from pH 5.3 to 5.7.

#### METHODS

The method described by Hissink (7) was adopted with very slight modification for this work. Enough air-dry soil to constitute a 25-gram sample of water-free soil, finely pulverized, was treated in an Erlenmeyer flask with 100 cc. of *N* salt solution heated to 70°C. This was shaken occasionally and allowed to stand over night. The whole was then brought quantitatively onto the filter and leached with successive portions of salt solution at room temperature until one liter of leachate was secured.

Preliminary determinations showed that the amount of  $\text{CaCO}_3$  in the soil samples was insufficient to introduce an appreciable error, a second liter of leachate in all cases containing only very small amounts of calcium. Ammonium chloride was used with the Crowley silt loam and sodium chloride of very high purity was used for all the Clarksville silt loam samples. Comparison of the two salts gave very good agreement in the results secured, the greater solvent action of the ammonium salt apparently largely offsetting the very small amount of calcium and magnesium in the sodium salt. Hence, no correction was made for the small amount of calcium and magnesium carried by the sodium salt.

Calcium was determined by the standard volumetric method and magnesium by a volumetric method described by Handy (5). This method for magnesium, though perhaps not quite so exact as the standard gravimetric method, gave satisfactory results and, requiring less time, enabled the writer to make a larger number of determinations than would have been possible otherwise.

#### EXCHANGEABLE CALCIUM AND MAGNESIUM IN AN OLD RICE SOIL (CROWLEY SILT LOAM)

The soil used in this study was taken from a number of plots of the Rice Branch Experiment Station at Stuttgart, Arkansas. Plots were selected which have never received fertilizer treatment, or at least have received none within the past 16 years. The samples were taken from soil which has been cropped to rice in all cases at least 15 of the past 18 years. The irrigation water used on all these plots comes from the same source, being from deep wells and containing considerable calcium and magnesium. The reaction of the irrigation water tested on two occasions during the last growing season was pH 7.3.

The results of the determinations on this soil are shown in table 1.

The results of table 1 are shown graphically in figure 1. The data differ from those for many soils in that the quantity of exchangeable calcium and magnesium is greater in the surface soil than in the underlying layer. The reason for this condition in the rice soil is undoubtedly the deposition of salts by the irrigation water in the upper stratum of the soil profile.

TABLE 1  
*Exchangeable calcium and magnesium in surface and subsurface horizons of a rice soil*

SAMPLE NUMBER, SURFACE SOIL	EXCHANGEABLE CALCIUM		EXCHANGEABLE MAGNESIUM		RATIO OF M.E. Ca TO M.E. Mg	SAMPLE NUMBER, SUBSOIL	EXCHANGEABLE CALCIUM		EXCHANGEABLE MAGNESIUM		RATIO OF M.E. Ca TO M.E. Mg	DIFFERENCE IN Ca OF THE TWO HORIZONS	DIFFERENCE IN Mg OF THE TWO HORIZONS
	Per cent	M.E.*	Per cent	M.E.			Per cent	M.E.	Per cent	M.E.			
101	0.0976	4.88	0.0254	2.09	2.33	201	0.0808	4.04	0.0182	1.50	2.69	0.0168	0.0072
102	0.1116	5.58	0.0259	2.13	2.62	202	0.0564	2.82	0.0110	0.90	3.13	0.0552	0.0149
104	0.1128	5.64	0.0220	1.81	3.11	204	0.0432	2.16	0.0096	0.79	2.73	0.0696	0.0124
105	0.1110	5.50	0.0264	2.17	2.53	205	0.0424	2.12	0.0081	0.67	3.16	0.0676	0.0183
106	0.1092	5.46	0.0225	1.85	2.95	206	0.0716	3.58	0.0206	1.69	2.11	0.0376	0.0019
107	0.1132	5.66	0.0220	1.81	3.12	207	0.0592	2.96	0.0096	0.79	3.74	0.0540	0.0124
15	0.1464	7.32	0.0355	2.91	2.51	18	0.0700	3.50	0.0192	1.57	2.23	0.0764	0.0163
16	0.1532	7.66	0.0350	2.87	2.66	19	0.0836	4.18	0.0220	1.80	2.32	0.0696	0.0130
17	0.1444	7.22	0.0292	2.40	3.00	20	0.0712	3.56	0.0177	1.45	2.45	0.0732	0.0115
21	0.1328	6.64	0.0321	2.64	2.51	26	0.1064	5.32	0.0244	2.00	2.66	0.0264	0.0077
24	0.1176	5.88	0.0316	2.59	2.27	28	0.1088	5.70	0.0273	2.24	2.54	0.0088	0.0043
25	0.1324	6.62	0.0321	2.64	2.50	29	0.1140	5.44	0.0250	2.05	2.65	0.0184	0.0071
Mean†	0.1234	6.17	0.0283	2.33	2.68	...	0.0756	3.78	0.0177	1.45	2.70	0.0478	0.0106
S.D.	0.0169	...	0.0047	...	0.089	...	0.0232	...	0.00607	...	0.316	...	...
C.V.	13.6	...	16.6	...	3.32	...	30.6	...	34.2	...	11.7	...	...
Odds	Infinite												

\* M.E. = milligram equivalents per 100 gm. of soil.

† Formulae used in statistical analysis were as follows:

$$S D = \sqrt{\frac{2D^2}{N}}$$

$$C V (\text{coefficient of variability}) = \frac{100 \times S D}{\text{Mean}}$$

$$P E (M) (\text{probable error of the mean}) = \frac{.6745 \times S D}{\sqrt{N}}$$

$$P E (\text{difference}) = \sqrt{E_a^2 + E_b^2}$$

$$\text{Odds (student's method using Love's tables)} Z = \frac{\text{Mean (difference)}}{S D}$$



The soil of the area from which these samples were taken appears to be extraordinarily uniform. The figures, on the contrary, indicate that there is probably considerable difference in the amount of leaching taking place, as the coefficient of variability for the subsurface samples in case of both bases, is

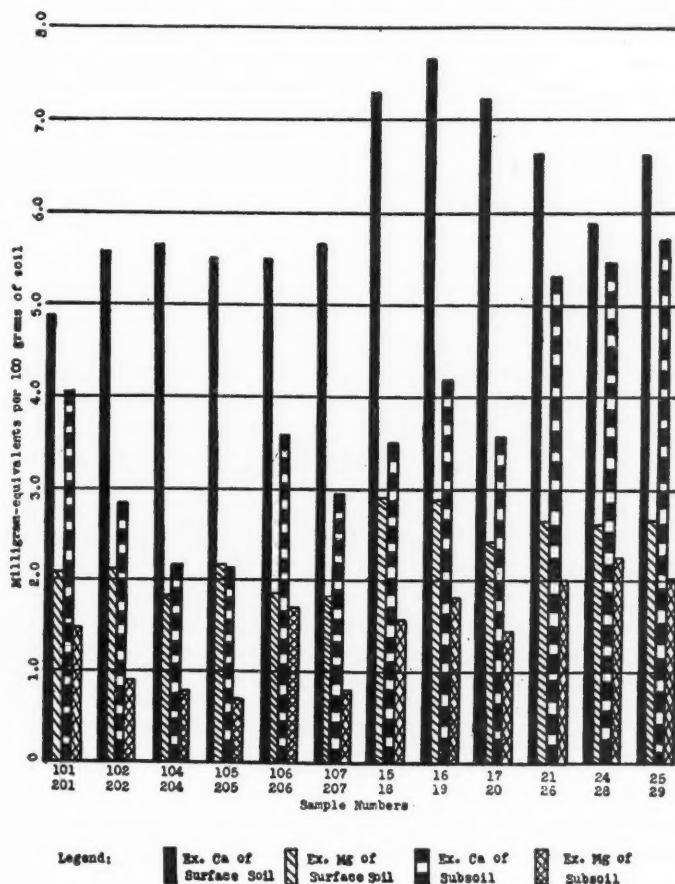


FIG. 1. EXCHANGEABLE CALCIUM AND MAGNESIUM IN TWELVE SAMPLES FROM EACH OF TWO HORIZONS OF AN OLD RICE SOIL

rather high. The impervious nature of the subsoil in general, however, is indicated by the relatively low content of exchangeable calcium and magnesium in most of the samples from the sub-surface horizon. The ratio of calcium to magnesium is rather remarkably constant throughout.

EFFECT OF MOISTURE CONTENT OF THE SOIL UPON THE EXCHANGEABLE  
CALCIUM AND MAGNESIUM

The ordinary procedure in rice production is to grow the crop in standing water for a period of about 75 days, beginning within 10 to 20 days from the time the plants emerge from the soil. Therefore, it seemed desirable to determine what effect submergence might have upon the exchangeable calcium and magnesium of the soil. This problem could not be studied through the use of field samples because such samples are modified by the salts carried in the irrigation water. Accordingly, a thoroughly mixed, screened and finely pulverized quantity of Clarksville silt loam was divided into 30 samples of 200 gm. each for laboratory experimentation. These samples were placed in wide-mouthed bottles and distilled water added to ten of them until it stood  $\frac{1}{2}$  inch above the surface of the soil. A second group of 10 samples received sufficient distilled water to bring the moisture content to 20 per cent, which is approximately optimum for the soil. The third group of 10 samples was left air-dry. The bottles were then weighed and stored where they were not exposed to the air of the laboratory. Sufficient distilled water to bring the samples up to weight was added once each week. After 75 days no more water was added and the soil was allowed to become air-dry. It was then finely pulverized and subjected to analysis. The results are listed in table 2.

When the results of these determinations are compared one group with another, some very interesting facts are revealed. Considering calcium first, the means of the columns for the air-dry and 20 per cent moisture samples show a difference of only  $0.0002 \pm 0.00045$  per cent, entirely insignificant. When the mean of the air-dry samples or of the 20 per cent moisture samples (the two being practically the same) is compared with the mean of the flooded samples, a difference of  $0.0104 \pm 0.00047$  per cent is obtained. This difference is 22.1 times the probable error and, hence, gives very high odds that it is significant. Thus, flooding the soil for a period of 75 days depressed the exchangeable calcium.

Now turning to magnesium and comparing the mean of the air-dry samples with that of the 20 per cent moisture samples, a difference of  $0.0012 \pm 0.00021$  per cent is obtained. This difference is 5.7 times the probable error and established a reasonably high degree of probability that 20 per cent moisture content caused a significant increase in exchangeable magnesium. Comparing air-dry samples with flooded ones a difference of  $0.0102 \pm 0.000435$  per cent is obtained, 23.4 times the probable error. The 20 per cent moisture samples compared with the flooded ones show a difference of  $0.0090 \pm 0.000435$  per cent in favor of the flooded ones. This is 20.6 times the probable error. Thus, it is apparent that flooding had opposite effects upon the exchangeable calcium and magnesium, depressing the quantity of the former and increasing the latter. The ratio between the two, therefore, is very decidedly narrowed. These results are shown graphically in figure 2.

TABLE 2  
*Exchangeable calcium and magnesium in Clarksville silt loam kept at various moisture contents for a period of seventy five days*

Sample number	AIR-DRY SOIL						20 PER CENT MOISTURE						FLOODED															
	Exchangeable calcium			Exchangeable magnesium			Ratio of M.E. Ca to M.E. Mg			Exchangeable calcium			Exchangeable magnesium			Ratio of M.E. Ca to M.E. Mg			Exchangeable calcium			Exchangeable magnesium			Ratio of M.E. Ca to M.E. Mg			
	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	Per cent	M.E.	M.E. Ca to M.E. Mg	
D1	0.0924	4.62	0.0086	0.71	5.79	1-20	0.0952	4.76	0.0096	0.79	4.85	1S	0.0832	4.16	0.0201	1.65	2.18											
D2	0.0912	4.56	0.0091	0.74	6.33	2-20	0.0924	4.62	0.0120	0.98	5.91	2S	0.0808	4.04	0.0206	1.69	2.56											
D3	0.0900	4.50	0.0086	0.71	6.16	3-20	0.0920	4.60	0.0108	0.89	5.59	3S	0.0808	4.04	0.0201	1.65	2.37											
D4	0.0916	4.58	0.0096	0.79	6.42	4-20	0.0932	4.67	0.0096	0.79	5.21	4S	0.0824	4.12	0.0158	1.30	2.85											
D5	0.0912	4.56	0.0086	0.71	6.50	5-20	0.0928	4.64	0.0108	0.89	5.21	5S	0.0804	4.02	0.0192	1.57	2.82											
D6	0.0920	4.60	0.0100	0.82	5.60	6-20	0.0908	4.54	0.0105	0.87	5.16	6S	0.0808	4.04	0.0225	1.85	2.36											
D7	0.0908	4.52	0.0091	0.74	6.10	7-20	0.0884	4.42	0.0108	0.89	4.71	7S	0.0804	4.02	0.0172	1.41	3.16											
D8	0.0908	4.52	0.0091	0.74	6.10	8-20	0.0884	4.42	0.0096	0.79	5.04	8S	0.0800	4.00	0.0206	1.69	2.39											
D9	0.0912	4.56	0.0091	0.74	6.10	9-20	0.0908	4.54	0.0110	0.90	5.28	9S	0.0820	4.10	0.0211	1.73	2.44											
D10	0.0924	4.62	0.0110	0.90	6.16	10-20	0.0920	4.60	0.0105	0.87	6.02	10S	0.0812	4.06	0.0177	1.44	2.52											
Mean.....	0.0914	4.56	0.0093	0.76	6.03	.....	0.0916	4.58	0.0105	0.87	5.30	.....	0.0812	4.06	0.0195	1.59	2.57											
S.D. ....	0.0007	.....	0.0007	.....	0.394	.....	0.00199	.....	0.0007	.....	0.403	.....	0.00096	.....	0.00192	.....	0.243											
P.E (M).....	±0.000149	.....	±0.000149	.....	±0.0841	.....	±0.000424	.....	±0.000149	.....	±0.08602	.....	±0.000204	.....	±0.000409	.....	±0.0518											
C.V. ....	0.76	.....	7.52	.....	6.53	.....	2.17	.....	6.66	.....	7.60	.....	1.18	.....	9.84	.....	9.45											

In order to further verify the results set forth in the preceding paragraphs, a group of soil samples was taken from jars in the greenhouse in which various rice experiments had just been completed. Clarksville silt loam had been used in these experiments. Four pairs of jars were sampled, each consisting of one jar in which the soil had been flooded and one in which similar soil was kept at optimum moisture content. Comparisons between jars of one pair and those of another were not possible, as the soil of the various pairs of jars

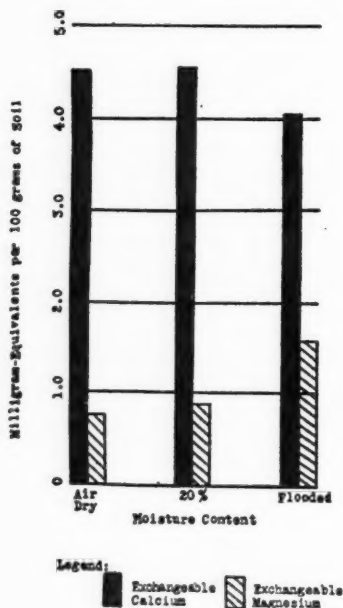


FIG. 2. EFFECT OF MOISTURE CONTENT UPON EXCHANGEABLE CALCIUM AND MAGNESIUM (Average of ten samples)

was not from the same source. The result of the analyses for exchangeable calcium and magnesium in these samples are given in table 3.

The results with the greenhouse soil samples show the same general effects of flooding, calcium being depressed and magnesium increased. There is one exception in the figures for magnesium, the sample of soil kept at optimum moisture content in one case showing more exchangeable magnesium than the flooded soil. The writer can offer no explanation for this exception.

THE EFFECT OF ONE SEASON OF CROPPING WITH CORN UPON EXCHANGEABLE  
CALCIUM AND MAGNESIUM

It would have been desirable in this work to have studied the effect of cropping with rice upon these bases, but material for such a study was not available. Rice does not require large amounts of calcium and since corn also does not draw heavily upon the calcium and magnesium of the soil, the effect of cropping with corn was studied. Cropped and fallowed plots with no fertilizer treatment were available for sampling. Nine pairs of such plots were sampled thoroughly and the composite sample from each plot mixed well and analyzed. The results are shown in table 4.

TABLE 3  
*Exchangeable calcium and magnesium in samples of greenhouse soil, flooded, and kept  
at optimum moisture content*

SAMPLE NUMBER	TREATMENT	EXCHANGEABLE CALCIUM		EXCHANGEABLE MAGNESIUM		RATIO OF M.E. Ca to M.E. Mg
		Per cent	M.E.	Per cent	M.E.	
278	No crop, flooded	0.1128	5.64	0.0235	1.93	2.92
288	No crop, optimum moisture	0.1216	6.08	0.0137	1.12	5.42
279	No crop, flooded	0.1129	5.64	0.0152	1.25	4.51
289	No crop, optimum moisture	0.1211	6.05	0.0126	1.03	5.87
234	Cropped,* flooded	0.0364	1.82	0.0104	0.85	2.14
238	Cropped,* optimum moisture	0.0550	2.75	0.0099	0.81	3.39
44	Cropped, flooded	0.0285	1.42	0.0104	0.85	1.67
46	Cropped, optimum moisture	0.0336	1.68	0.0126	1.03	1.53

\* Cropped twice, successively with rice.

These results do not indicate any measurable effect of cropping with corn for one season, as compared to fallowing, upon the exchangeable calcium and magnesium. The mean differences obtained are supported by insignificant odds. To add further evidence on this point, however, a pair of plots was selected which had been subject to a certain amount of what appeared to be very uniform sheet erosion. These were selected because it was believed they would be low in exchangeable calcium and magnesium and thus small differences might be more readily measured. Ten samples were removed from the plot which was maturing a good crop of corn and 10 from the fallowed plot. The results secured with these samples are given in table 5.

When the results for calcium in table 5 are compared, the difference of the means is found to be  $0.0025 \pm 0.0079$  per cent. The difference is, therefore, less than the probable error and hence, not significant. Comparing the results for magnesium, a difference of the means of  $0.001 \pm 0.00056$  per cent is found,





TABLE 5  
Exchangeable calcium and magnesium in a fallowed plot and a plot cropped to corn for one season

CROPPED PLOT										
Sample number	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg	Exchangeable calcium		Exchangeable magnesium		Ratio of M.E. Ca to M.E. Mg
	Per cent	M.E.	Per cent	M.E.		Per cent	M.E.			
F1	0.0836	4.18	0.0096	0.78	5.35	0.0688	3.44	0.0091	0.74	4.64
F2	0.1056	5.28	0.0086	0.70	7.54	0.0936	4.68	0.0096	0.78	6.00
F3	0.0460	2.30	0.0081	0.66	3.47	0.0548	2.74	0.0076	0.62	4.41
F4	0.0420	2.10	0.0052	0.42	5.00	0.0360	1.80	0.0062	0.50	3.60
F5	0.0272	1.36	0.0062	0.50	2.72	0.0336	1.68	0.0086	0.70	2.40
F6	0.0880	4.40	0.0072	0.59	7.45	0.1128	5.64	0.0128	1.05	5.37
F7	0.0360	1.80	0.0052	0.42	4.28	0.0404	2.02	0.0057	0.46	4.34
F8	0.0404	2.02	0.0062	0.50	4.04	0.0380	1.90	0.0076	0.62	3.06
F9	0.0680	3.40	0.0057	0.46	7.39	0.0412	2.06	0.0048	0.39	5.28
F10	0.0360	1.80	0.0052	0.42	4.28	0.0280	1.40	0.0057	0.46	3.04
Mean.....	0.0572	2.86	0.0067	0.54	5.15	0.0547	2.73	0.0077	0.63	4.21
S.D.....	0.0256	....	0.00149	....	1.66	0.0269	....	0.00226	....	1.108
P.E.(M).....	±0.00546	....	±0.000318	....	±0.354	±0.0057	....	±0.00046	....	±0.236
C.V.....	44.7	....	22.2	....	32.2	49.1	....	29.3	....	26.3

favoring the cropped soil. This difference, however, is less than twice the probable error and it, also, is not significant. These results, therefore, are in accord with those previously presented and indicate that one season of cropping with corn produced no measurable effect upon the exchangeable calcium and magnesium of the soil. The selection of plots for this study prove a rather unfortunate one as both plots, though only 1/20-acre in size, showed high variability.

#### DISCUSSION OF RESULTS PRESENTED

The figures obtained for the old rice soil, Crowley silt loam, reveal no unusual characteristics of the soil, except the rather infrequently encountered situation in which the greater concentration of the exchangeable bases is found near the surface. The deposition of salts by the irrigation water in the surface soil due to restricted leaching, is responsible for this condition.

The explanation for the depressing effect upon the exchangeable calcium produced by flooding the soil and its building-up effect upon the exchangeable magnesium is much more difficult. It has been shown by Subrahmanyam (18) that keeping a soil saturated with water reduces its hydrogen-ion concentration. He presented data, also, which showed that the decrease in hydrogen-ion concentration was concomitant with the increase in free and saline ammonia. The writer, working with Janssen (15) also observed this fact in rice soils, and found that most of the decrease in hydrogen-ion concentration disappeared when the soil was dried for a few days previous to sampling. It is rather difficult to see how this change could appreciably affect the exchangeable calcium and magnesium, particularly in divergent manner. If the flood water had been decanted or the soil solution displaced before the samples were analyzed, the decrease in calcium might be attributed, in part at least, to passage of a portion of the exchangeable calcium into solution. This would still leave the increase in exchangeable magnesium to be explained. The flood water was allowed to dry in the soil, however, and the soil solution was not displaced before the samples were treated with the salt solution. Gedroiz (4) found a very small increase in exchangeable calcium in soil which stood submerged in  $\approx$  NaCl solution for 3 months and for 14 months, but this, of course, represents very different conditions than flooding with distilled water. Martin and Christie (13) waterlogged a sandy loam soil and found that the concentration of calcium and of magnesium in the soil solution was depressed.

The writer is unable to offer an explanation for the divergent behavior of these bases in flooded soil. It would seem, however, that the phenomenon might be more easily explained if it is assumed that the exchangeable bases are held in the soil by true chemical forces, rather than to assume the double-layer type of adsorption proposed by Hissink. Submergence affected the soil colloids, and it required nearly one-third more time to leach the flooded soil samples than the corresponding samples kept air-dry or at optimum moisture content. Such a change in the physical condition of the soil might be advanced

to explain the decrease in exchangeable calcium, but it is difficult to see how the increase in magnesium could be explained on this basis.

Considering the fact that a corn crop which would yield 60 bushels per acre would remove perhaps not more than 2 per cent of the amount of calcium held in exchangeable form in the soil studied, (and in many cases, probable, less than 2 per cent) it is not surprising that no effect of cropping could be measured. The results are in accord with those secured by Martin (12), though his results are of greater significance because the periods of cropping and fallowing are much longer.

An interesting feature of the results secured with Clarksville silt loam is the rather wide ratio of calcium to magnesium. Most of the soils of this country upon which reports have been published show ratios approaching more nearly that of the Crowley silt loam.

#### SUMMARY

The results of a study of the exchangeable calcium and magnesium of a soil cropped to rice for a long period of time are reported in the foregoing paragraphs. The effect of flooding a soil, as in rice fields, and the effect of a season of cropping with corn upon the exchangeable calcium and magnesium were also studied.

Crowley silt loam cropped to rice for 15 of the past 20 years showed considerably more exchangeable calcium and magnesium in the surface soil than in the sub-surface horizon.

Keeping Clarksville silt loam at a moisture content of 20 per cent with distilled water for a period of 75 days increased the exchangeable magnesium as compared to the same soil kept air-dry. Exchangeable calcium was not appreciably changed. Flooding with distilled water for the same period of time depressed the amount of exchangeable calcium, but increased the amount of exchangeable magnesium.

Cropping with corn for one season brought about no measurable change, when compared with fallowing, upon the exchangeable calcium and magnesium of Clarksville silt loam.

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# SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: I. HISTORICAL AND INTRODUCTORY

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The influences which higher plants may exert upon development of the microscopic population of the soil and the effects which such microörganisms may have are naturally numerous. The fact that plant growth continues in soil and that microörganisms develop in this habitat are ample verification of this fact. Neither group of organisms can develop long in the absence of the other in nature without showing abnormalities due to the absence of the other associate. Most of the soil organisms depend upon the organic and inorganic plant residues as sources of food and higher plants depend upon the conversion of their own remains by microörganisms into assimilable substances as sources of nutrients. Inhibition of activities of either group of organisms eventually leads to modified development of the other. The reciprocal effects of the development of higher plants in intimate relationship with microörganisms are associated with a multitude of reactions occurring in the soil wherever higher plants grow. The activities associated with the complex transformation of organic and inorganic substances are largely regulated by microbial development. Starting with large mineral aggregates with which plant residues are incorporated the microörganisms play very important rôles in developing a disintegrated admixture of fine particles capable of supporting higher plants and furnishing to them the nutrients, either inorganic or organic, which are necessary for their maturity.

The plants withdraw considerable amounts of substances (principally inorganic) from the rhizosphere, modify the soil structure by the penetration of roots, exert solvent effects by the excretion of carbon dioxide and eventually introduce large amounts of organic matter to the soil in the form of their dead tissues which undergo disintegration through the agency of the microörganisms. These changes all affect the development of the soil organisms. In a comprehensive way it is difficult to distinguish between those activities of plant growth which do and do not affect microbial development and equally difficult to determine what activities of microörganisms in soils do not exert direct or indirect effects upon plant growth.

Close to the root systems the reactions of soil microörganisms may not be qualitatively different from those at a distance from the roots, but they may, and undoubtedly do, differ quantitatively due to the influences of the higher

plants and further, their effects upon plant development become apparent more quickly since their action occurs nearer to the organs of absorption of the higher plants.

#### HISTORICAL

Results of studies concerned with the influences of development of higher plants upon the abundance of groups of microorganisms or microbial activities in soils about root systems may be interpreted as reflecting the resultant effect of a variety of influences of the plants upon the rhizosphere, partly depressive and partly accelerative. Among the various modifications of the soil which the plants may provoke are the following: lowering of the concentration of certain mineral nutrients in the soil due to their absorption, partial desiccation of the soil by absorption of water, increase in soil carbonates following root excretion of carbon dioxide, contribution to microbial foods by sloughed off root portions and excretions. Some of these effects may directly affect the soil population and others indirectly by modifications of the physical composition of the soil. The attraction of phytophagous insects and worms about roots may eventually lead to influences upon the microorganisms.

Knowing what marked changes are brought about in soils by desiccation (26, 23) and what influences such effects may exert upon the biological properties (67), it is not surprising that combinations of a variety of environmental changes should result in modifications of the biological activities.

Rokitskaia (51) observed that decomposition of cellulose proceeded at different rates in soils obtained from the root zones of various plants. The explanation of this effect may appear from an appreciation of the nutrition of the soil organisms which naturally require considerable abundance of mineral nutrients and available nitrogen to decompose cellulose. The different degrees of exhaustion of the nitrogen and other necessary substances by root absorption may explain the observed effects on cellulose decomposition.

*Microorganisms penetrating the plant.* The most striking development of microorganisms about roots of higher plants appear in cases of actual penetration of roots by the microorganisms. This may result in pathological conditions where the microorganisms cause marked destruction of cells and tissues; it may result in passive conditions with little or no injury; or it may be a condition of actual beneficial effects of the invader upon the host. Several classes of this last case are known: 1) the association of bacteria with legumes, 2) mycorrhiza formations with such plants as woody trees, orchids and heathers (49, 40), 3) possible cases of stimulation to root development (50), 4) invasion of many non-legumes by passive or symbiotic bacteria called "bacteriorrhiza" by Perotti (47, see also 57, pages 447-460).

Penetration of roots of most plants including annuals and perennials, woody and herbaceous is probably the rule rather than the exception (20). The extent of the penetration and degree of the effects vary with the soil conditions which determine the vigor and degree of resistance. Under favorable cultural



conditions there may be no apparent injury but, under adverse conditions of moisture, temperature, or nutrient supply the invader may cause definite injury.

*Influence of inorganic root excretions upon soil organisms.* Excretion of both organic and inorganic substances by plant roots appear of particular interest in their influences upon microbial activities as well as in nutrition of the plants themselves. Although it is apparent that higher plants must absorb more of the inorganic substances than they excrete, still at certain stages of growth, there may be appreciable elimination of certain substances both organic and inorganic, nitrogenous and non-nitrogenous.

Fred and Haas (13) verified the observations of Sachs (52) and Knop (24) that roots may exert a solvent effect upon limestone by the action of the carbon dioxide which is liberated. They further extended these observations by demonstrating that the presence of microorganisms enhanced this solvent action. This suggests that the microorganisms may exert pronounced effects about roots as a result of their ability to produce carbon dioxide presumably from the decomposition of organic root excretions. Stoklasa and Ernest (58, 59) and Aberson (1) showed that the strong corrosive action was due to carbon dioxide and that other acids played no important rôle under normal conditions of plant growth. Czapek (11) and Wilfarth, Römer and Wimmer (71) observed that appreciable amounts of inorganic compounds of potassium, calcium, magnesium, phosphates, sulfates, and chlorides may be eliminated by the plant roots into the soil. Stoklasa (58, 59, 55), Kunze (25) and others (see 41, 17) have shown that the carbon dioxide evolved from roots and by microorganisms greatly facilitates absorption of essential elements from relatively insoluble soil minerals. Newton (43, 44) emphasizes the importance of excretion of carbon dioxide by plant roots in the feeding power of plants. The results of Parker (45, 46) show that there may be no correlation between amounts of nutrients absorbed and carbon dioxide evolved by the roots of higher plants growing in the presence of an abundance of soluble nutrients (as in cultural solutions). However, in the soil environment, the carbon dioxide evolved from roots and by the soil organisms may play an important rôle in determining the ability of higher plants to absorb nutrients. The consumption of oxygen and formation of carbon dioxide in the soil both from microbial action and from plant roots may exert pronounced effects upon root development (7, 8).

Neller (42) observed that the carbon dioxide produced from soil supporting growth of higher plants was appreciably greater than the amounts produced by unplanted soil. In explaining his results it seems necessary to assume that the plant growth actually must have accelerated the decomposition of the soil organic matter by effecting a more thorough aeration or some other similar action. Lundegårdh (30) concluded that the contribution of the microorganisms to the carbon dioxide of the atmosphere generally far exceeds that of the roots of higher plants under normal conditions. Stoklasa's results

(57) would lead to a similar conclusion. Barakov (2) and Turpin (62) concluded that the plant often produces, at the period of its most active growth, many times as much carbon dioxide as is produced by soil organisms, and that the excess of carbon dioxide in the soil growing a crop is due to respiratory activity of the plants rather than to the decay of root particles from the crop growing upon the soil. That this conclusion may not be entirely justified may appear from the experimental results to be reported. Metzger (41) found the soil bicarbonates in much greater concentration near plant roots than at a distance from the roots. The greatest increase in bicarbonates appeared at the period of heading of the plants. What portion of these effects may be attributed to the soil microorganisms is speculative.

Headden (17) also observed a marked increase in carbon dioxide in soils under growing plants and attributed to a large degree the beneficial influence of one crop upon subsequent ones to the solvent action which this carbon dioxide exerts upon insoluble soil minerals. Due to the fact that the amount of carbon dioxide in the soil decreased to a low level following the cutting of a crop it was concluded that the growth of higher plants suppresses microbial activities and that the increase in carbon dioxide found under plants is due to excretion of the gas from the plant roots. These conclusions are not in accord with facts observed by others regarding the development of microorganisms in the rhizosphere (21, 27, 68, 29, 53, 16).

*Influence of organic root excretions upon soil organisms.* Little is known as to the nature or abundance of excretions of organic substances from roots. Lyon and Wilson (35) determined the amounts of residues and excretions from plant roots developing in solutions free from microorganisms. These solutions showed the presence of organic nitrogen even before the nitrate nitrogen had all been absorbed. In some cases there appeared to be a decrease in the amount of organic nitrogen with progressive stages in the life of the plant, especially at the period close to maturity. The ratio of organic matter in solution to that in the plant was from 1-37 to 1-65. The organic matter contained relatively small amounts of nitrogen. Cranmer (9) found a considerable abundance of phosphatides excreted by roots of higher plants, seeds and seedlings. It was believed by Melin (39) that such materials play an important rôle in favoring development of mycorrhiza fungi about roots leading to their subsequent penetration of the roots and associative development with the plants.

The much discussed problem of soil toxins, and effects of one drop upon another may find interpretation from information concerning root excretions, absorption by roots and residual products of plant disintegration. Associative effects of plants and microorganisms find expression in the modification of the abundance of cells of different physiologic groups of microorganisms about roots and their activity in the rhizosphere. Beijerinck (4) found larger numbers of *Azotobacter* in soil close to roots of legumes than in soil from roots of non-legumes. It may be logical to consider that organic plant

residues about plant roots would serve as a suitable source of food for nitrogen fixing organisms consequently favoring their development and associated fixation of nitrogen. In fact, Truffaut and Bezssonov (61) claim to have grown corn to maturity in media containing no nitrogen but containing nitrogen-fixing bacteria. It was believed that the organic matter excreted from the plant roots served as food for the bacteria, which fixed the nitrogen, which in turn became available to the plants. Such results as these should be repeatedly verified before being seriously accepted as indicating the facts. It seems unlikely, however, even though small amounts of nitrogen were fixed under such conditions, that they would be sufficient to satisfy the demands of the plants. Further, since the nitrogen-fixing bacteria were the only ones present, it seems unlikely that any appreciable amount of the fixed nitrogen would become available to the plants. At least, it is known that, under natural conditions where the same agencies should appear active,

TABLE 1  
*Influence of plant development upon abundance of bacteria and formation of carbon dioxide (Stoklasa)*

SOIL DEPTH	PASTURE		LUCERNE		WHEAT		SUGAR BEETS	
	Bacteria	CO <sub>2</sub>	Bacteria	CO <sub>2</sub>	Bacteria	CO <sub>2</sub>	Bacteria	CO <sub>2</sub>
	cm. millions	mgm.*	millions	mgm.*	millions	mgm.*	millions	mgm.*
10-20	2.300	16.5	54.800	60.6	24.000	47.5	38.700	56.4
20-30	2.500	19.4	52.500	62.8	25.600	49.7	41.200	58.2
30-50	0.140	9.8	14.800	46.2	26.000	28.5	22.000	36.4
50-80	0.012	3.3	0.770	16.3	2.200	6.6	5.500	8.3
80-100	0.005	2.2	0.082	3.7	0.049	4.3	0.200	4.8

\* From 1 kg. soil in 24 hours.

non-legumes require the presence of an abundance of nitrogen which is not supplied through the agency of non-symbiotic nitrogen-fixing organisms.

Joshi (21) showed that non-legumes might benefit from associative development of nitrogen-fixing bacteria although no nodulation developed.

*Influence of plant development upon abundance of soil microorganisms.* Velich (63) found an organism called *Clostridium gelatinosum*, Laxa. in greater abundance in soils close to the roots and upon the root epidermis of beets, mangels, barley, rye, oats and wheat than at a distance from the roots. Similar observations were also made by Maassen and Gruber (see Löhnis, 28, p. 16). Hoffman (18) reported that, in 27 out of 32 cases investigated, the numbers of bacteria in soils adjacent to roots were much greater than in soils at a distance from the growing roots. Further studies demonstrated that development of different plants exerts different effects upon any one organism and that development of different organisms may exert a variety of effects upon any one plant.

LeClair (27) found the bacteria more abundant in planted than in un-

planted soil. Johnson and Levine (19) found certain gram negative bacteria (coli-like) in greater numbers in planted soils. Greaves, Stewart and Hirst (61) found the numbers of organisms more abundant in cropped than in fallow soil. Stoklasa's results (56) show a rather close correlation between the abundance of bacteria in soils under different plants and the evolution of carbon dioxide from these soils. Further, these differences were apparent not only in the surface layers of soil but persisted even in the deep layers (table 1). The fact that roots of weeds evolved greater amounts of carbon dioxide than cultivated plants led him to believe that such plants had a greater feeding power and consequently could frequently compete to advantage with cultivated plants.

Somewhat larger numbers of nitrogen-fixing bacteria and total bacteria were found in planted than unplanted soils by Joshi (21). Legumes favored the development of the greatest abundance of organisms.

Wilson and Lyon (68) grew plants under controlled conditions in sterilized soils inoculated with pure cultures of bacteria with the results that much greater numbers of bacteria developed in practically all cases as compared with development in the absence of plant growth.

The results of Löhnis (29) show that the numbers of bacteria increased as a result of plant growth, particularly in the case of some of the legumes. The response to the crops was much greater the second year. This might be expected in view of the fact that continuous cropping leaves residues which should keep the abundance of organisms at a relatively high level; an uncropped soil, however, undergoes progressive depletion of microbial foods and consequently decreases in biological activity. The numbers of *B. radiobacter* fluctuated, but in general were more numerous under the legumes. The actinomycetes and filamentous fungi were slightly higher under plants than in the absence of plant growth. The general increases in the soil population persisted even after the periods of harvesting the crops but a thorough desiccation of the soil caused a more or less complete disappearance of the effects.

Creuzburg (10) attacked the problem in the identical way followed by Löhnis but obtained less striking results. Although some increase in numbers of bacteria was observed under legumes, even a decrease was found under cereals. Even second plantings of crops gave very slight differences under cereals. He concluded that only certain plants under certain conditions exert an influence upon numbers of soil bacteria. No significant influence of the stage of growth of the plants upon the abundance of organisms was apparent from the results of either Löhnis or Creuzburg.

Smith (53) observed that growth of legumes increased the numbers of *B. radiobacter* from the third week after planting and this effect persisted some weeks after harvesting the crops. Although the results in the field were less striking than in the greenhouse, the abundance of these organisms was greater nearer the roots, often none being found one foot distant from the roots.

Taranovskaia and Germanov (60) believed that the increases in numbers

of bacteria and acceleration of nitrate formation by growth of clover was due to improvement in the physical structure and physico-chemical properties of the soil by plant growth. From the results of Batchelor and Curie (3) there appears to be a correlation between crop yields (and consequently root residues) and abundance of nitrogen-fixing anaerobes in soils. The greatest number of the organisms appeared in soils supporting the best development of higher plants.

*Influence of plant development upon nitrification.* The rapidity of accumulation of nitrate nitrogen in soils need not be associated with the abundance of nitrifying organisms. Even though such organisms occur in abundance and even though considerable ammoniacal nitrogen be added, nitrate nitrogen may not accumulate for a considerable period of time in case any abundance of decomposable non-nitrogenous organic matter be present.

King and Whitson (22) found that nitrates accumulate in soil more rapidly after growth of clover than corn or oats (see also Löhns, 28, p. 766-770). Lyon, Bizzell, B. D. Wilson and J. K. Wilson (31, 32, 33, 34, 37, 38, 69, 70) noted that greater amounts of nitrate nitrogen accumulated under legumes than non-legumes and that nitrification subsequent to removal of the crops was greater from the soils which had grown legumes. Brown (5, 6) found that clover in rotations favored nitrification. Greaves (15) however, reported that nitrification in alfalfa soil was lower than that in wheat soil. Although it would appear from Löhns' results (29) that nitrification proceeded more rapidly under plants than where no plants were growing, laboratory tests failed to indicate that plant development modified the nitrifying capacity of the soils. However, the method used for estimating this reaction could hardly be expected to detect such differences.

It was concluded by Lyon and his co-workers that the striking effect of legumes was associated with the nitrogen content of the root residues from the plants; the non-legumes were believed to introduce into the soil, organic matter of lower nitrogen content than the legumes. Roots added to soils have been shown to depress nitrate formation in direct order of the percentage content of nitrogen in the roots (36). Information from many sources shows that the consumption of nitrogen in the nutrition of soil microorganisms, developing upon non-nitrogenous organic matter, determines the extent of nitrification (12, 48, 54, 65 and 66 chapter 26).

Lyon and Bizzell (33) found that, in the early stages of growth, plants accelerate nitrification but that at the periods of more advanced development they depress nitrification. Cropping, in general, but cropping to legumes in particular favored nitrification. This enhanced ability of the alfalfa soil to form nitrates persisted for more than a year after the crop was removed. Greaves, Stewart and Hirst (16) observed that plant growth favored the nitrification process.

In general, it appears justifiable to consider, from the information available, that development of higher plants results in modifications of the soil popula-

tion and that marked increases in the abundance of certain microorganisms frequently occur. Certain activities associated with these organisms are also consequently affected.

#### EXPERIMENTAL PROCEDURE

If it were to be expected that development of higher plants would exert effects upon the soil organisms it seemed reasonable to assume that pronounced effect would be most likely to appear when plant development was well advanced. Preliminary observations were consequently made of soils supporting a variety of plants in the fall of the year. The plants used and the condition of the plants at the time of sampling were as follows:

Sugar beets—plants well developed with heavy roots, vigorous vegetative growth, plants in condition to be harvested.

Alfalfa—second cutting of plants six months old, vigorous vegetative growth.

Field corn—plants approaching maturity, kernels still somewhat soft, plants still green.

Eggplant—had been yielding fruit for several weeks, vegetative growth still green.

Rye—fall cover crop about six weeks old, about eight inches high, vigorous vegetative growth.

Apple tree—about ten years old, fruit had already been harvested this season, leaves still green.

Samples of soil were collected from as near to the roots as possible and in some cases, as with the sugar beets, the soil was taken from the roots subsequent to removing the plants from the soil. At the same time, samples of soil were taken about a foot distant from the plants at the same depth as sampled from the roots but taken so as to include as few roots as possible or none at all. The soils were passed through a 3 mm. sieve and thoroughly mixed before studying.

The following biological observations were made upon these soils:

1. Determination of the abundance of colonies of filamentous fungi developing upon Waksman's acid agar (64). In preparing this medium for use, instead of adjusting the reaction of the medium before sterilization, the acid was added just before pouring the plates. Before sterilization, the medium was titrated with 0.2 *N* acid and calculations were made to determine how much acid should be added to 300 cc. of the medium to bring the reaction to pH 3.8. The medium was sterilized in 300 cc. portions in 500 cc. Erlenmeyer flasks. Standard 0.2 *N* H<sub>2</sub>SO<sub>4</sub> was sterilized in a separate flask at the same time. At the time of pouring the plates, when the medium was melted the calculated amount of acid was added with a sterile graduated pipette. Adjustment of the reaction by this means insured a firm medium. Such a method of adjusting the reaction would permit lowering the pH below that possible where the acid was added before sterilization.
2. Determination of the abundance of colonies of actinomycetes developing upon Waksman's albumin agar (14, medium 5).
3. Determination of the abundance of colonies of bacteria developing upon the albumin agar.
4. Determination of the abundance of colonies developing upon nitrogen-free mannite agar (14, medium 77).



5. Determination of the abundance of mucoid colonies developing upon the nitrogen-free mannite agar.
6. Determination of the carbon dioxide evolved in fifteen days from portions of soil calculated to be the equivalent of 100 gm. oven-dry soil.

For the plate counts five plates were counted at the dilution at which the soils were plated. Only averages of these counts are reported.

#### EXPERIMENTAL RESULTS

The results of these observations are presented in table 2 and figure 1. In the figure, the unshaded columns are extended below the zero line to represent the abundance of organisms and evolution of carbon dioxide in the soils obtained from a distance from the plant roots. The black columns represent the results of the measurements made upon the soils obtained from the rhizosphere. These black columns extend as far below the zero line as the unshaded columns. Consequently any extension of the black columns above the zero line represents the detection of greater numbers of organisms or greater evolution of carbon dioxide in the soil near the plant roots than in the soil distant from the roots. The greater the extension of the black columns above the zero line the greater the determined abundance of organisms or evolution of carbon dioxide in the soils about the roots of the plants concerned.

It is quite apparent that all of the groups of organisms studied appeared in greater abundance in the soils close to the plant roots than in similar soils devoid of root development. These differences appear to be of such an order as to be significant in all cases. Differences are evident between the extent of the influences of root development upon the different groups of organisms and also upon the influences of different plants upon any single group of organisms. It is not intended that these results should be accepted as reflecting the influences which these various plants may exert upon the soil population at any period during the growing season. On the other hand, it is very apparent from the results which will appear in subsequent papers that the plants exert very different effects upon the soil population at different stages in their development. These data however, would seem to indicate that, at any one time, particularly when the plants are well advanced in growth, the microörganisms are more numerous in the soil which has been penetrated by the plant roots.

Of all the organisms studied, the fungi appeared to be least affected by root development. The increases varied between 6 per cent and 80 per cent. The average increase in fungi under all of the plants was 39 per cent. Although these differences may be considered to be significant, the determination of fungi by the plate method is not sufficiently accurate to permit the assumption that slight differences are more than suggestive.

The actinomycetes appear to be affected to a somewhat greater extent than the fungi. The increases due to development of plants varied between 23 per cent and 275 per cent. The average increase under all plants was 115



TABLE 2  
*Influence of plant development upon microorganisms in the soil*

PLANT	REGION OF SAMPLING	FILAMENTOUS FUNGI		ACTINOMYCES		BACTERIA		TOTAL COLONIES ON MANNITE		MYCOID COLONIES ON MANNITE		CO <sub>2</sub> EVOLVED IN 15 DAYS	
		Numbers	Increase due to plants per cent	Num- bers millions	Increase due to plants per cent	Num- bers millions	Increase due to plants per cent	Num- bers millions	Increase due to plants per cent	Numbers × 100,000	Increase due to plants per cent	Amounts mgm.	Increase due to plants per cent
Apple tree.....	Near roots	88,000	57	3.2	129	17.4	195	17.6	329	22.8	2,750	11.0	214
	Away from roots	56,000	..	1.4	..	5.9	..	4.1	..	0.8	..	3.5	..
Rye.....	Near roots	216,000	33	4.4	38	28.6	117	16.0	191	22.4	300	8.9	19
	Away from roots	162,000	..	3.2	..	13.2	..	5.5	..	5.6	..	7.5	..
Corn.....	Near roots	178,000	33	13.4	52	41.0	69	35.8	92	23.4	117	18.2	52
	Away from roots	134,000	..	8.8	..	24.3	..	18.6	..	10.8	..	12.0	..
Sugar beet.....	Near roots	222,000	26	15.0	23	57.8	80	53.8	63	29.8	176	17.5	84
	Away from roots	176,000	..	12.2	..	32.1	..	33.0	..	10.8	..	9.5	..
Egg plant.....	Near roots	144,000	80	9.0	275	85.0	445	47.6	198	27.0	34	12.5	112
	Away from roots	80,000	..	2.4	..	15.6	..	16.0	..	20.2	..	5.9	..
Alfalfa.....	Near roots	268,000	6	9.0	173	93.8	427	63.0	189	116.0	616	20.4	146
	Away from roots	254,000	..	3.3	..	17.8	..	21.8	..	16.2	..	8.3	..
Average increase due to plants (per cent).....		39	..	115	..	222	..	177	..	666	..	..	105

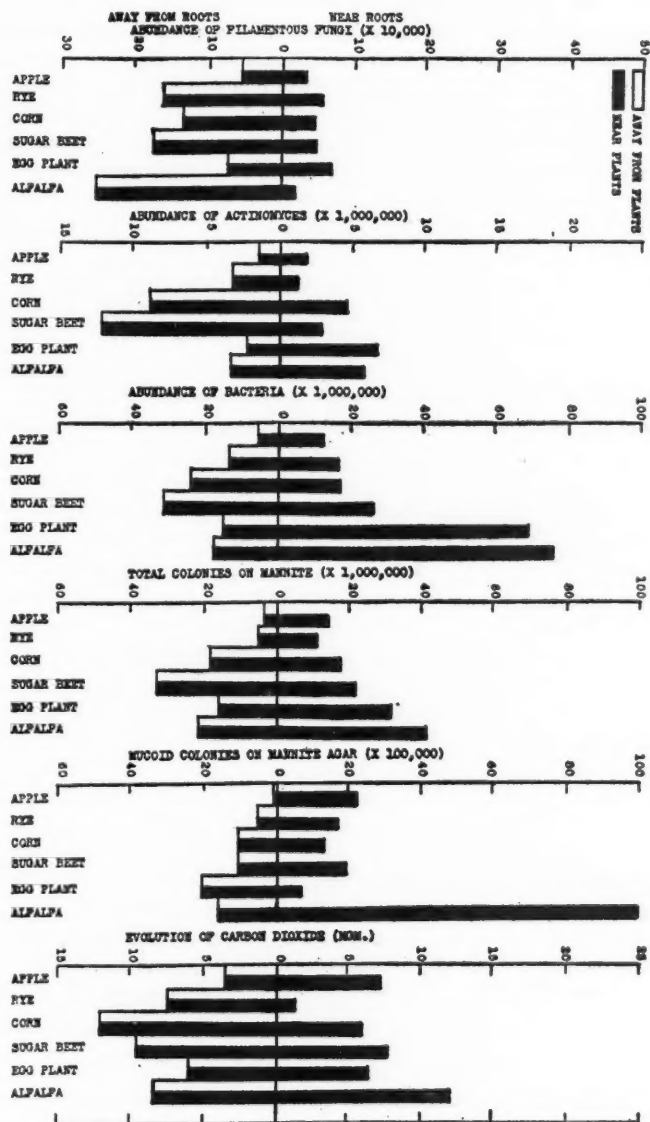


FIG. 1. INFLUENCE OF DEVELOPMENT OF PLANT ROOTS UPON THE ABUNDANCE OF MICRO-ORGANISMS IN SOIL AND THE FORMATION OF CARBON DIOXIDE

per cent. The plate method of determining the abundance of actinomyces in soils may be considered as reflecting the abundance of these organisms in the soil more accurately than such a measurement of fungi.

The numbers of bacteria developing upon albumin agar are affected to a striking degree by plant development. The increases in numbers varied between 69 per cent and 445 per cent. The average of the increases under all plants was 222 per cent. The total colonies developing upon nitrogen-free mannite agar represent much the same organisms as occur upon albumin agar and do not represent nitrogen-fixing bacteria. The effects of plant development upon the abundance of these organisms is much the same as the effects upon the bacteria developing upon albumen agar.

The mucoid colonies developing upon the nitrogen-free mannite agar include such organisms as *B. radiobacter*, *Aero. aerogenes*, *B. radiculicola* and related forms which are non-spore-forming, gram-negative rods which may or may not fix nitrogen. This group was increased to a greater degree by root development than any of the other groups of organisms studied. Although the percentage increase was greatest about the roots of the apple tree, by far the greatest increase in numbers of the organisms appeared about the roots of alfalfa.

The only measurement made of the biological activity in these soils was the evolution of carbon dioxide. This is a more exact measurement of the biological condition of soils than the enumeration of any of the organisms by plate counts, consequently, slight differences in formation of the gas may be considered to be significant where similar differences in plate counts would seem to be within the error of the determination. In all cases considerably greater amounts of carbon dioxide were formed by soils obtained from plant roots than by soils supporting no root growth. The average increase due to plant growth was 105 per cent.

It is suggestive that the rye exerted the least influence upon formation of carbon dioxide of all the plants studied, and that the apple roots exerted the greatest proportional increase. This may appear more suggestive in view of the fact that the rye plants were very young while the roots of the apple tree had been developing in the soil over an extended period of years.

These data as a whole suggest that higher plants may affect certain groups of organisms to a different degree than others and that the order of the extent of the influence of growth of different plants upon different groups of organisms may not be the same.

#### SUMMARY

Material is presented introductory to a series of observations upon the influences of development of higher plants upon the microbial population of soils. The preliminary experimental observations indicate that microorganisms occur in greater abundance about plant roots than at a distance from the roots. Such appeared to be the case with all of the organisms studied:

filamentous fungi, actinomycetes, bacteria developing upon albumin agar, organisms developing upon nitrogen-free mannite agar, and, the group of bacteria related to *B. radiobacter* which develop as mucoid colonies upon nitrogen-free mannite agar. Different plants affected any one group of microorganisms differently and caused greater proportional and absolute increases in the abundance of certain groups of organisms than others. Organisms related to the *B. radiobacter* group, bacteria developing upon albumin agar and organisms developing upon nitrogen-free mannite agar increased to a greater degree as a result of plant growth than did the actinomycetes or filamentous fungi.

Soils supporting root development produced much more carbon dioxide than soils devoid of roots.

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